HEMATOLOGICAL INVESTIGATIONS IN ETHIOPIA

with special reference to a high iron intake

by

YNGVE HOFVANDER

UPPSALA 1968

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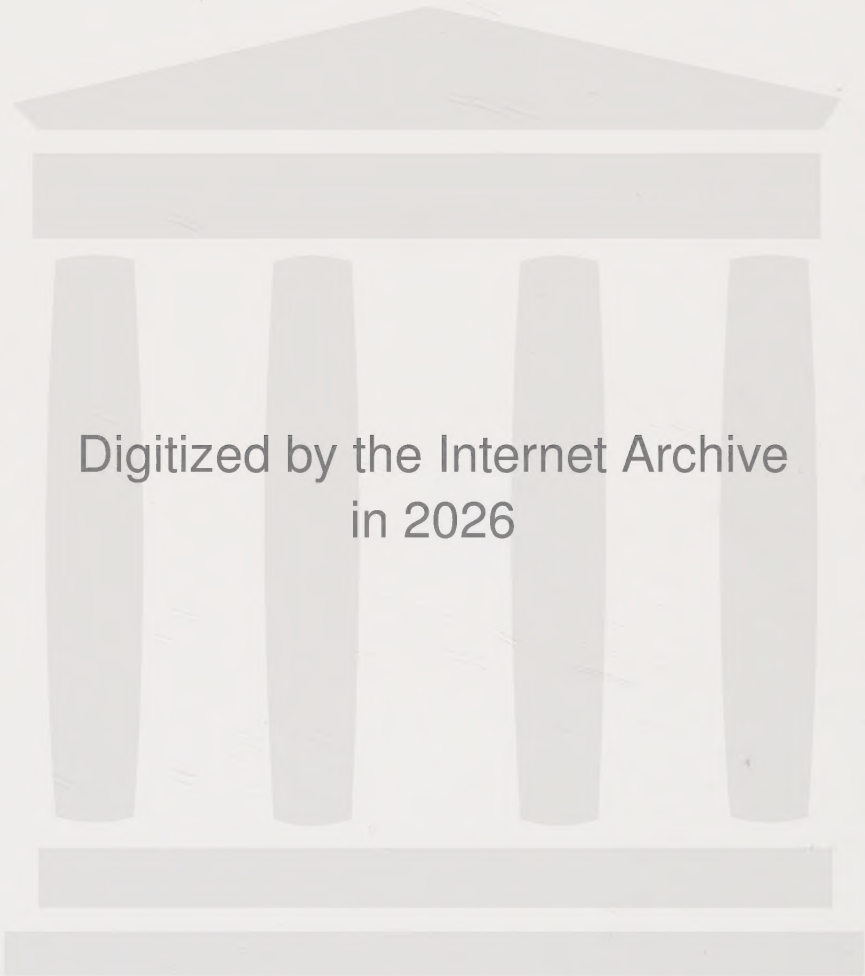
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Abbreviations

Hb	Hemoglobin concentration
PCV	Packed cell volume, hematocrit
MCHC	Mean corpuscular hemoglobin concentration
SI	Serum iron
TIBC	Total iron binding capacity
ESR	Erythrocyte sedimentation rate
CNU	Children's Nutrition Unit
ESPC	Ethio-Swedish Pediatric Clinic
ICNND	Interdepartmental Committee on Nutrition for National Defense

CNU report No. 19

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Preface

Aim of the present study

In 1958, the Interdepartmental Committee on Nutrition for National Defense (61) made a country wide comprehensive dietary and clinical survey in Ethiopia. One of the very interesting discoveries during this study was the enormous dietary intake of iron, amounting to several hundred mg daily in the Ethiopian highlands where the cereal tef constitutes the staple grain. It was concluded that iron deficiency anemia was no serious problem and low hemoglobin values were recorded in only one place (Gambella) which had a high frequency of parasitism and was situated at a low altitude. The high dietary iron intake has later been confirmed by others (101). In view of the comparatively high altitude at which these investigations were performed the mean Hb values obtained in these studies, especially in adults, would be considered rather low, suggesting a suboptimal material. Additional studies were therefore considered desirable. It also seemed of interest to determine whether the often excessive dietary intake of iron led to a saturation of the iron stores to an extent similar to what has been described in the Bantus.

The aim of this investigation was thus to study

1. the sources of the food iron in the tef diet on the Ethiopian highlands;
2. certain hematological values (Hb, PCV, ESR, SI and TIBC) in healthy and apparently healthy subjects of different ages with a high iron intake at a medium high altitude and to try to obtain a reference standard and to estimate the prevalence of anemia among these subjects;
3. the iron stores in various age groups by examination of bone marrow hemosiderin, by desferrioxamine tests and by chemical liver iron determination on an autopsy material;
4. the frequency and types of anemia in an Ethiopian village (children as well as adults) with a high dietary iron intake, as measured by simple tests, Hb, PCV, ESR and in subsamples SI, TIBC, serum folic acid and vitamin B 12. Previous studies (77) in the village chosen, which is representative of the rural conditions on the Ethiopian highlands, had indicated a rather high frequency of anemia.

ETHIOPIA
Field centres and stations belonging to the CHILDREN'S NUTRITION UNIT
▲Field centre
■Field station
• Major town
Asmara
TIGRÉ
Gondar
SUDAN
Dessie
FRENCH SOMALIA
SOMALIA
IJAJI
ADDIS ABABA
Harar
ARUSSI
SIDAMO
KENYA
0 100 200 300 400 500
Kilometres

CHAPTER I

The country

Ethiopia is located in the "Horn of Africa", immediately north of the Equator. It is about 2½ times the size of Sweden and has a population variously estimated at 20—24 milj.

Almost two-thirds of the empire is a mountainous highland plateau, surrounded by regions of desert, savannah, lowland and expanses of volcanic rock. Over 90 % of the people live in rural areas, primarily by a subsistence type of agriculture and animal husbandry (106). The population is composed of many tribes and several languages are spoken. The official language is Amharic which is the mother tongue of about 1/3—1/4 of the population.

The medical and health facilities include government and mission hospitals as well as mission clinics and governmental health centers and health stations. In 1966 there were 70 hospitals (7,751 beds), 95 mission clinics, 63 health centers and about 480 health stations (16). The country as a whole had only about 370 physicians in 1966; all but 42 were foreigners employed on a more or less temporary basis. All other health staff is in equally short supply and to a great extent concentrated to the capital and other urban areas.

Due to incomplete vital statistics there is no reliable information as regards morbidity for the country as a whole. However, tuberculosis, malaria, leprosy, yellow fever, helminthiasis, liver cirrhosis and, especially among children, malnutrition and intestinal infections are among the most commonly met diseases.

The infant mortality rate has been studied in a few communities (106, 111) where it has been found to range between 86 and 196 per 1,000 live births.

Children's Nutrition Unit

The Children's Nutrition Unit (CNU) was started in the fall of 1962 with the following major aims according to the agreement between the Ethiopian and Swedish governments:

> "To undertake a survey of the incidence of malnutrition in selected groups of Ethiopian children."
>
> "To make a detailed analysis, chemical and biological, of the food consumed in the families under study."
>
> "To start an enrichment program with special emphasis on locally available indigenous foodstuffs."
>
> "To make an evaluation of the physical fitness in relation to nutritional status in school age children and adults."
>
> "To participate in nutrition teaching on various levels."

The project was planned by a group of Swedish scientists, who had been working on problems related to nutrition for many years: Professor Bo Vahlquist (chairman), Professor O. Mellander, Professor G. Ågren and Assistant Professor G. Almgård, who succeeded the late Associate Professor P. E. Nilsson. The committee works in an advisory capacity to the Swedish International Development Authority (SIDA) and the project, and members of the group usually spend a working period yearly in Addis Ababa.

The base institution contains a well equipped laboratory for biochemical work and food analysis, an experimental kitchen, rooms for production of supplementary mixtures, stores and offices for the different departments. The CNU is also in charge of a Children's Home nearby, where supplementary foods and weaning foods are tested.

The total staff consists of about 10 foreigners and 70 Ethiopians.

During the years 1963—1965 field surveys were performed in 5 different localities in the country, selected in cooperation with a socio-anthropologist, to be representative of the living conditions of large population groups. In two of these places (Addis Ababa and Ijaji, a village 220 km west of the capital) a long term study of the growth, development and health condition of the children from 0—10 years was performed while in the other three, cross sectional studies were made (77). In addition to clinical examination, samples of blood, urine and stools were analysed and dietary surveys were made. It is estimated that the five test areas are representative of more than half the population of Ethiopia. The information and data thus obtained, especially those from the dietary surveys, have been used in an applied nutrition program with special emphasis on teaching nutrition on various levels. This program has been extended successively since 1965 as a weapon in the combat against the widespread malnutrition.

Weaning food recipes, utilizing ingredients commonly used by the people, have been worked out and tested and are now being spread through available channels, including radio.

A supplementary feeding program started in late 1965. A number of mixtures had been worked out based on amino acid analysis of the (vegetable) protein ingredient and on biological testing (1). After acceptability tests on staff members and orphanage children, a large scale supplementary feeding program was launched including several thousands of children. This proved quite successful and one of these supplementary mixtures was marketed under the name "Faffa" ("grow strong and healthy") in the spring of 1967.

SIDA has so far provided most of the funds for the project. The Ethiopian Government has provided counterparts to some of the foreign staff members. UNICEF has contributed machinery for the production of "Faffa" as well as dried skim which is included to 10 % in the mixture.

An important aspect of the work is to train Ethiopians to take over in due time. Scholarships have thus been provided for several of the senior Ethiopian staff members to study abroad and in-service training is going on systematically (117).

CHAPTER II

Dietary iron on the Ethiopian highlands

The source of the iron

Introduction

The main staple crop on the Ethiopian highland is tef, *Eragrostis abyssinica*. As far as is known Ethiopia is the only country that uses tef as a cereal crop, though it is cultivated for its hay in other parts of Africa (76). The seed is sown at the end of the rainy season (June—September) and it is harvested in November—December, traditionally by cutting the straws by hand with a sickle. By that time the soil is very dry and dusty. The threshing is done by making oxen walk around in the straws, thereby expelling the grain. This is done on a hard surface of soil and cow dung. The grain is separated from the chaff by the time-honoured method of throwing them up into the air on windy days. The seeds are swept together and put into sacks.

Before being milled the grain is cleaned. A few handfuls are put on a strawplate and by small revolving movements of the plate combined with blowing, most of the visible contamination is discarded.

After milling (traditionally stone mills) the flour is mixed with water, a piece of the previous dough is added and the mixture is left to ferment for 1—3 days, after which a sourbread, enjera, is baked.

The individual tef grain is very small, only the size of a pinhead. The weight of 1,000 seeds is 0.3—0.4 g as compared with about 50 g for wheat (4). The relative surface is consequently very large and it has also minute husks, which makes it difficult to clean completely.

There has been some confusion, however, as to the question of whether the iron is *in* or *on* the tef grain. This may be of importance as regards the absorbability of iron. The ICNND report (61) states that... "The exceedingly high content of iron (approximately 100 mg of iron per 100 g of tef) may reflect some contamination of the cereal, but all samples analyzed as well as the prepared foods show this high iron content. Analysis of tef washed with acid to remove adhering soil, etc., indicates that less than 10 per cent of the iron is due to contamination".

Russel (quoted by Postmus (92)) found a mean value of iron of 90 mg/100 g and ICNND (61) 105 mg/100 g in tef "as purchased".

Almgård (4) cleaned the grain mechanically under a magnifying glass and found that the samples contained about 1 % soil which could be separated. Analysis of tef cleaned in this way showed iron contents of 5.2 and 5.9 mg/100 g of dry matter for red and white tef, respectively. This would correspond to values found in wheat and barley.

Melak Mengesha (76) had 12 white and 12 red tef strains grown in a field in Indiana, USA, and carefully avoided soil and iron contamination when harvesting. He found that the iron content was 2—3 times higher in tef than in wheat, barley or grain sorghum (10.6—19.6 mg/100 g of dry matter for uncontaminated seed), and is not inclined to accept Almgård's low figures.

Own studies

It seems important to differentiate between the iron in tef "as purchased" which usually has much soil contamination and the tef iron determined after various cleaning methods. Some studies have been carried out at CNU to this end.

The iron content in the reddish soil is high. In the CNU field center in Ijaji, 9 soil samples from different tef fields were analysed for iron according to the same method as used for analysis of food iron (Schade *et al.* (98), modified by K. Jacobsson (64)). The percentage of iron in these samples ranged between 5.36 and 7.32. Water from two different wells in the village contained 0.14 and 0.47 mg iron per liter, while the tap water in Addis Ababa (1 sample) contained 0.30 mg per liter, suggestive of a low solubility of the soil iron. The amount of iron in soils apparently varies from country to country but an average of 3.8 % has been reported (24).

Sergegna tef (red and white mixed) was purchased from 3 different sources (100 kg each). All had been harvested by hand. One was threshed by machine, and the other two in the traditional way by cattle. The three samples were all cleaned at CNU by machine sifting and different kinds of contamination separated. The result is seen in Table 1.

Table 1. *Various kinds of contamination (% of weight) in 3 different tef qualities, bought at the local market, Addis Ababa.*

	Straw, stones, excrements	Soil, dust, grit	Weeds, husks	Total
Sergegna tef Aroussi 1st quality Machine threshed	0.82	0.25	0.51	1.58
Sergegna tef Debre Zeit 1st quality Animal threshed	1.34	0.15	0.40	1.89
Sergegna tef unknown origin, lowest quality and price Animal threshed	5.67	0.52	2.3	8.49

It is evident from these figures that the iron content of tef "as purchased" can be strongly influenced by contamination, which in the lowest quality tef totalled 8.49 % of the weight.

An attempt was also made to determine the iron content in tef "as eaten" after different stages of mechanical cleaning and washing. Samples of white, red and mixed (sergegna) tef, grown in the village Ijaji (CNU field center) were sifted and cleaned by hand in the traditional way (= tef "as eaten" in the true sense).

Subsamples of this were then washed with iron-free water and hydrochloric acid (about 2 %) 20 times and again analysed for iron according to the previously mentioned method. The result is seen in Table 2.

Table 2. *Iron content in different types of tef after traditional cleaning and after further washing 20 times in dilute hydrochloric acid.*

	mg Fe/100 g tef* after traditional cleaning	mg Fe/100 g tef* after further washing × 20 in dilut. iron free HCl
White tef	57.9	13.0
White tef	79.4	36.9
White tef	35.8	14.5
Red tef	103.0	20.0
Red tef	97.0	24.8
Mixed (sergegna) tef	60.0	11.5
Mixed (sergegna) tef	30.1	27.4
Mixed (sergegna) tef	25.1	12.4

* dry weight. Moisture in tef about 10 %

Generally the iron content was reduced to between 1/2 and 1/4 of the "as eaten" values after the washing procedure, indicating that most of the iron in tef "as eaten" is derived from soil contamination, which is contrary to what is stated in the ICNND report (61). A comparison between results of different investigators is given in Table 3.

As can be seen the figures of the present author are in good agreement with those of Melak Mengesha. The red tef gave the highest iron values in both these studies, a little more than 20 mg per 100 g non-dried tef. This is approximately 4 times greater than the iron content in wheat or barley (27). It would be tempting to conclude that the beautiful red color of this tef strain is derived from a high iron content, higher than in the white tef.

Table 3. *Iron content in "clean" tef, according to 3 different investigators.*

	mg Fe/100 g of dry matter in cleaned seed (Almgård 1963) (4)	mg Fe/100 g of dry matter in uncontaminated seed (Melak Mengesha 1964) (76)	mg Fe/100 g of dry matter, acid washed seed (present study)
White tef mixed strain	5.9	11.5	13.0 14.5 36.9
Red tef mixed strain	5.2	19.6	20.0 24.8
Red, white mixed (Sergegna)	5.5	15.5	11.5 12.4 27.3

Two values for iron in washed tef, one white tef and one mixed, are in considerable disaccordance with others of the same tef type. It is possible that some soil contamination may still adhere to the grain in spite of careful washing. On the other hand there is also a possibility that there are genetically different strains with different iron contents. This cannot be fully excluded in view of the varying results of different investigators.

However, the important thing is that tef which is by far the most common staple food on the highlands has a high iron content in the "as eaten" form.

Determinations of soil iron were made from two more places in the Rift valley about 200 and 300 km south of Addis Ababa (at CNU field stations). In one (near Lake Zwai) the iron content was considerably lower than in Ijaji, viz. 1.51 % (1 sample) while in the other (near the provincial capital of Sidamo) it was comparable to that in Ijaji — 4.83 and 5.14 % (2 samples).

The important point, however, is that tef is not grown in these areas (mainly corn, sorghum and ensete, the false banana). The high degree of contamination is firmly connected with tef, due to the special harvesting procedure, and the size of the grain, where in addition small husks facilitate the adherence of small particles of soil.

Dietary iron intake

Ethiopia may represent the country with the highest intake in the world of iron per person per day, even greater than that of the Bantu natives in South Africa. ICNND (61) found an average iron intake per person per day of 471 mg with a range of 98—1418 mg. Dietary surveys (101) and food analyses (1) performed at CNU revealed the following as regards iron intake. An adult man eats about 2—3 enjeras per day, each baked of 200—225 g tef. Adult male shoe factory workers in Addis Ababa (5) had a mean iron intake of 520 mg daily with a range of 250—870 mg. The consumption of iron in adult males was of the same order in the village Ijaji. In government school children aged 9—11 and 12—14 years in Addis Ababa (5) a dietary survey (recall method) indicated an iron intake of 346 and 420 mg (range 121—730 mg) while for the same age groups in a private French school (Ethiopian children) it was a little lower, 272 and 329 mg, respectively (range 5—680 mg) due to a smaller consumption of tef enjera. In Ijaji in children from 1/2 year to about 5 years the iron intake ranged between 1 mg to about 50 mg, depending upon the amount of tef enjera consumed (101).

In general in the ordinary adult diet in tef areas, the major part of the iron intake is

derived from tef while the remainder comes from spices, mainly berberi, and legumes (101). ICNND (61) found in a typical Ethiopian diet with about 2,500 calories and an iron intake of 433 mg, that 405 mg was derived from tef and 28 mg from other food sources. Contributions from the traditional cooking utensils (clay pots), although not specifically investigated, are probably of little significance.

If it is assumed that an adult male eats 2½ tef enjeras per day, each baked of 200 g red tef with an iron content of 100 mg per 100 g (Table 2 — "after traditional sifting" = tef "as eaten"), the iron intake from this source would be 500 mg. If, further, it is assumed that the figures for the iron content in red tef given by Melak Mengesha ("uncontaminated seed") and those presented in this study (seeds washed 20 times with dilute HCl) — approximately 20 mg per 100 g — really represent the iron *in* tef, then about 100 mg iron would be consumed from the tef itself. The corresponding figure for white tef and mixed, sergegna tef would be a little lower. Using Almgård's figures in this example the total daily iron intake from tef itself would be lower, probably depending on the analytical method giving too low values. In recent investigations on tef cleaned under a magnifying glass Almgård found higher iron values than previously reported (personal communication).

The availability for absorption of the iron on or in the tef has not been studied, although there are reasons to believe that for the soil iron which is usually present as complex oxides and hydroxides, it is probably not high (24).

Even if only a small percentage of iron from the contaminating soil were available for absorption the high total amount ingested would guarantee a reasonable daily supply. In any case the iron in the tef grain itself seems to provide a substantial daily iron intake, which is considerably above the "recommended allowance" (94).

CHAPTER III

Hematological values in healthy and apparently healthy subjects of different ages in Addis Ababa, 2,400 m. above sea level

Introduction

During the course of nutrition surveys performed by CNU over the years 1963—1965 (77) and in investigations made in the village Ijaji before the start of an applied nutrition program in 1965 (56) it became evident that a substantial part of the child population had marginally low hemoglobin values or frank anemia in spite of a high iron intake found in dietary surveys. The need for a reference standard was often felt. It appeared, however, that there were two main obstacles in obtaining such a "normal" child material. The first was to get healthy non-infected subjects of different ages and who were in a satisfactory state of nutrition and the second was to overcome the traditional fear of blood letting.

As for adults it was considered that Ethiopian staff members of CNU and ESPC with easy access to medical attention if necessary and with a stable although for certain groups low income would be a satisfactory reference.

An opportunity to obtain a hematological reference standard in children arose when examinations of presumably "optimal" children in the private French school were planned, to get an anthropometric reference standard. To this material of adults and school children, a series of pregnant women and newborn children were also added. As it was not possible to obtain samples in the "1st class" ward or "1st class antenatal clinic" they were taken in the ordinary "3rd class" clinic.

The fear of blood letting is deep rooted and seems to depend upon the assumption that blood is not regenerated. Even the small amounts of capillary blood used in finger prick tests caused a great stir in the village Ijaji and the whole base line examination in November 1965 was near to collapse when in addition a rumour was spread that the blood drawn was sold in Addis Ababa. A common misbelief was also that the (Ethiopian) staff drank the blood from the people — apparently after they had been seen pipetting the samples. The author has experienced several times that vital blood transfusions could not be given because the (compatible) father refused to give even 100 ml to his child with the statement that he himself would die, get seriously ill or lose his potency. On the other hand cautery, bleeding, cupping and the use of leaches is still quite widely practiced traditionally against various diseases (87). With increasing education this traditional fear is diminishing. The Vacutainer technique facilitated matters as the blood obtained by venipuncture could be concealed in the tube by the examiner's hand or by a paper cone.

Material

Adults. The material consisted of 87 healthy and apparently healthy males and 50 apparently healthy non-pregnant females, most of them staff members of CNU and ESPC or in a few cases employed by foreign staff members of CNU. 19 maintenance workers at the ammunition factory in Addis Ababa were also included.

There were different kinds of professional and educational backgrounds. There was the "professional" staff such as administrators, nurses, student nurses, dressers, laboratory technicians, field assistants, qualified nutritionists and nutrition workers — all with secondary school education or more and all comparatively well paid according to local conditions. There were also unqualified or "non-professional" staff mem-

bers with lower salaries and low or no formal school education, such as ward dressers, cleaners, assistants in the food production department, guards, maintenance workers and the like. The majority of the "professional" staff were unmarried and were in a position to spend more of their (higher) salary on better food or, as in the case of the student nurses, had their meals at the hospital.

Blood samples were drawn from these subjects during the periods January—April and November—December 1967 (dry season). All were volunteers and all were in good health without symptoms of infection in the preceding weeks. Most of them had had a lung X-ray shortly before. The majority of the males and about 1/3 of the females had a yearly health check by the staff doctor at CNU, and most of the males were given a brief physical examination on the day of the blood testing to rule out infections and other ailments. All had daily access to medical attention if needed.

Children. During the periods May—June and November—December 1967 a total of 402 children aged 1—14 years inclusive underwent blood tests. The children of school age (374) were students at the French school, one of the very best in the country as regards standard of teachers and education and attended by some 1,500 students, of whom about 70 % are Ethiopians. It is a "status school" with a fee which is high enough according to local conditions to place the "socially privileged" in great majority although a liberal number of scholarships are granted.

The blood tests were made as part of a survey for obtaining an anthropometric reference standard for Ethiopian children. All Ethiopian children of ages 4 years (kindergarten) and upwards were examined clinically and anthropometric measurements taken. According to a pre-decided number system, all children with a last digit of 2, 6 and 9 were chosen for blood tests (Hb, PCV, ESR). If a child with one of these digits happened to be absent on the day of examination the next child was chosen for blood tests. No child refused. The remaining 28 children, most of whom were in the age group 1—5 years were called for examination. Their parents were socially privileged and also the mother had secondary school education or more.

In the French school venous blood for SI and TIBC was taken only from the children examined in November 1967. Of the others, venous blood, also for SI and TIBC, was taken from all whenever this was technically possible (a few small children were missed).

In no case did physical examination of the blood-tested children reveal any signs of infection requiring treatment or absence from school. In this respect, as well as in other respects, the children differed markedly from a general child population in the country (see chapter V).

The two materials mentioned were put together and the sexes combined. 57 of the children were of ages 1—4 years, 230 were 5—9 years and 115 were 10—14 years. The number of children in the peripheral parts of the age span 1—14 years was fairly small. Only 14 children were below 3 years of age and only 17 children were 14 years (7 girls).

The ages of the children were obtained through a questionnaire filled in by the parents, in which also other social data such as ethnic group, parents' professions etc. were given. With few exceptions the birth month as well as the birth year was given and was presumably correct. At the time of examination the child was also asked about his or her age. In the vast majority of cases the child gave the same information as the parents. In a few cases there was a discrepancy of one year in which case usually the examining physician decided on the spot which age was the most likely. Possible errors in age determinations will be minimized by the age grouping of the children.

Pregnant women, mothers at delivery and newborn infants. During the periods January—March and November—December 1967 blood samples were obtained from 52 pregnant women in the 7th—9th month of gestation at the "3rd class" antenatal clinic at Princess Tsahai Memorial Hospital in Addis Ababa. Some had minor complaints, related to the pregnancy. Most of the women attending this clinic are delivered at the hospital in due time and thereby represent a low middle class who can afford the delivery fee of Eth. $ 15, plus $ 2 per day plus cost of medicines, laboratory tests etc.* The vast majority even in Addis Ababa are delivered at home attended by a local "midwife" and with no antenatal or postnatal care.

About half the women attending the clinic start to come for checking at about or even before the 4th month of gestation but variations from as early as 6 weeks to as late as "early labor" occur. The women are blood grouped according to ABO and Rh and all have a hemoglobin determination at the first visit. Only in cases of moderate to gross anemia is iron medication prescribed. This applies to less than 5 %. About 2,000 deliveries take place yearly at the hospital (Dr. R. H. J. Hamlin, personal communication).

In the fall of 1967 cord blood (for SI and TIBC) was obtained from 47 newborn infants with a birth weight of more than 2,500 g. An attempt was made to obtain blood samples from the respective mothers also just before delivery, but this was not possible due to a heavy strain on the ward staff, especially at night. With respect to the newborn infants, an attempt was made to use the cord blood collected not only for SI

* 1 Eth. $ = 0:40 US $.

and TIBC but also for Hb etc. However, the cord blood collected in heparinized Vacutainer tubes clotted in nearly all cases, presumably partly because the tubes were not shaken properly and partly because the heparin may have whirled away when the stopper was removed from the vacuum tube. Finger prick blood for determination of Hb and PCV therefore had to be taken from 30 other infants, with a birth weight of more than 2,500 g, within the first 36 hours of life (Nov.—Dec. 1967).

From 27 women, venous blood was obtained during the last few hours before delivery except in one case when it was taken 3 hours after delivery.

The collection of blood from the women in the antenatal clinic was made in consecutive cases one morning weekly; at the delivery department this principle could not be upheld completely due to shortage of staff. All newborn infants not older than 36 hours, available 2 mornings a week, were tested consecutively.*

Methods

All blood samples collected were taken, except in the newborns, from the cubital vein with the subject sitting; disposable needles were used. From the newborns, capillary blood for determination of Hb and PCV was taken after a finger prick and for SI and TIBC from cord blood, which was allowed to drip freely from the placental part of the cord into iron-free tubes. Blood from children 1—14 years old and from adults was taken as follows: after short or no stasis a disposable needle for Vacutainers was inserted in the vein and a 10 ml Vacutainer tube (iron-free without additives) was filled with blood. Within seconds, about 1 ml of this blood was poured over to an Ellerman tube and pipetted for determination of Hb, PCV and ESR. Repeated checks showed that the whole procedure took only about 20 seconds after training and with 2 assistants taking care of the blood for pipetting. During a period when no Vacutainers were available, the blood from children in the French school and from adults was collected in two different tubes, the first 1 ml in an Ellerman tube, immediately taken care of for determination of Hb, PCV and ESR and, with the disposable needle still in place, a second 10 ml iron-free tube was filled for SI and TIBC determinations. The time needed for this procedure was equally short.

Blood from pregnant and delivering mothers was collected in two different Vacutainer tubes, one with heparin with a 72-hour anticoagulant effect (for Hb and PCV) and one iron-free without additives (for SI and TIBC). Immediately before pipetting, the heparin tube was carefully and slowly rocked about 20—25 times to homogenize the blood again. The maximal time between blood taking and processing for Hb and PCV was 2 hours.

All blood for determination of SI and TIBC was centrifuged after clotting, and the serum usually deep-frozen until processed after 1—2 weeks.

All blood samples for determination of SI and TIBC were taken between 9 and 11 a.m., except in delivering mothers and from cord blood. The subjects were not fasting as this was not possible from a practical point of view. The morning meal is, however, very light and in most cases consists of tea with sugar and white bread without butter or cheese. Only exceptionally was the colorimetric reading disturbed by turbidity.

Hemoglobin (Hb) was determined as cyanmethemoglobin. 25 µl of blood was mixed with 5 ml of ferricyanide-potassium cyanide solution (prepared fresh weekly from Metrix® dilutent tablets, Michael Reese Research Found., Chicago, Ill., USA, according to instructions) and read in a Beckman C spectrophotometer at 540 mµ.

The instrument was calibrated at regular intervals. The error of a single determination as calculated** from 30 consecutive duplicate determinations on capillary blood was 0.32 g % (the mean value of these reading was 11.73 g %).

Hematocrit (PCV) determinations were made according to the instruction manual for the M.S.E. micro-hematocrit centrifuge. Commercially available, heparinized capillary tubes were used in duplicate. The tubes were sealed at one end in a hot flame and centrifuged for 5 minutes. According to the instruction manual the centrifuge should make 10,000 rev/min. The reading was made in a hematocrit reader and the mean value of the readings of the 2 tubes was recorded. It was seldom possible to seal and centrifuge the blood filled tubes until after 1/2—3 hours. For various practical reasons the tubes could not be sealed with plastelina which gives a more sharp demarcation of the lower end of the packed cell column as compared to a more

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* I am very grateful to Professor C. Rendle-Short and to Drs. R. H. J. Hamlin and C. Nicholson for permission to make these examinations and to the ward staff for their assistance.

** The calculation was performed according to the formula:

$$\sigma_i = \sqrt{\frac{\Sigma d^2}{2n}}$$

where d is the difference between duplicate determinations and n the number of differences.

U-shaped bottom in flame sealed tubes. On the same blood sample, 10 hematocrit determinations with flame sealed tubes and 6 with plastelina sealed tubes were made. The mean value with the former method was 52.0 % and with the latter 52.5 %, a non-significant difference. In individual cases in the field work, however, the slightly unsharp demarcation may have caused minor reading errors. The "plug" of white cells at the other end of the red cell column was not to be included in the hematocrit reading. As the dividing line is not always distinct, minor reading errors may have been made in the field work. No correction was made for trapped plasma.

The error of a single determination as calculated from 20 consecutive duplicate determinations on capillary blood was 1.20 PCV %. The mean value for these readings was 39.2 %.

As has been pointed out recently (13) hematocrit readings tend to give too high values when centrifugation of the tubes is not done at once, when correction for trapped plasma is not made, when the white cell layer is included and when careful mixing, resulting in oxygenation, is not carried out. All these precautions were not or could not be taken in the field, for practical reasons. This resulted, in general, in slightly too high readings, which should be taken into consideration when comparisons are made with other materials.

Sedimentation rate (ESR) was determined according to the micro method described by Ström (108), using 3.7 % sodium citrate solution as anticoagulant. The mixture was drawn into the micropipette within 3 hours after the blood was taken. The temperature in the room was recorded.

The sedimentation rate increases with a rise in the environmental temperature (69, 74) while marked variations in barometric pressure, ultraviolet light and humidity do not seem to have any influence (69).

The temperature in the laboratory in Addis Ababa where the sedimentation rate was recorded for healthy adults and children was between 20 and 23° C while in Ijaji it ranged between 20 and 28° C on different days and at different times of the day. Most recordings were made there at about 24—25° C. At this comparatively low temperature there is only a very minor increase of the ESR, in the order of 1—2 mm (69). In the ESR recordings no correction was therefore made for the small increments which may have been caused by a slightly raised temperature.

Serum iron (SI) and total iron binding capacity (TIBC) were determined according to a method described by Schade *et al.* (98), slightly modified by Jacobsson (64).

In each set of tests, analysis of a standard serum (Nyegaard lot no. 3) was made simultaneously. In 41 such controls the mean and SD* for serum iron was 172.0 ± 8.56 µg %. This mean value is very close to the mean values obtained at 3 Scandinavian reference laboratories for this serum (64). For TIBC it was 311.8 ± 13.5 µg %.

Double determinations were made on sera from 25 consecutive subjects for SI and from 21 sera for TIBC. The errors of a single determination were 3.26 and 5.26 µg %, respectively. The mean value for SI was 120 µg % and for TIBC 313 µg %.

Altitude adjustment of "normal values"

There is an unsatisfactory state of knowledge concerning the precise alterations in peripheral blood values which occur with change of altitude, especially where medium high altitudes are concerned. Thus published investigations on "normal values" from altitudes approximately 1,500—3,000 m above sea level differ considerably (47, 86, 119).

Presumably this is so because of heterogeneous materials and differences in techniques. Hurtado *et al.* (57), in a detailed study, investigated several series of male subjects at sea level and at different altitudes up to 4,835 m and found that in general the level of polycythemia was directly proportional to the degree, duration and continuity of the anoxic stimulus. There seems to be a limit for the hematological response to the anoxic stimulus and, when this is extremely severe, a decrease rather than a further increase is observed in the resulting polycythemia. This appears to occur when the arterial oxygen saturation reaches a value of 60—70 %, corresponding roughly to an altitude of 6,000 m (57). The blood volume was found to be increased due to an increased mass of red cells, whereas the plasma volume remained unchanged or decreased. There was a tendency to slight macrocytosis, but as the hemoglobin content of the red cells was elevated

* The definition of standard deviation in this paper is

$$SD = \sqrt{\frac{\Sigma (x - \bar{x})^2}{n-1}}$$

correspondingly, the mean corpuscular hemoglobin concentration remained unchanged. The polycythemia was accompanied by a proportional elevation in the circulating reticulocytes and in the serum bilirubin, while the leucopoietic activity was not affected. The polycythemia observed in persons just arriving at high altitudes seems to be due to a release of stored blood and hemoconcentration, while the polycythemia corresponding to repeated or constant exposure to a low barometric pressure is a consequence of erythropoietic hyperactivity and is adjusted to the degree of anoxemia.

There was a striking inverse relationship between the degree of arterial oxygen saturation and the level of hemoglobin concentration in the circulating blood (see Fig. 1).

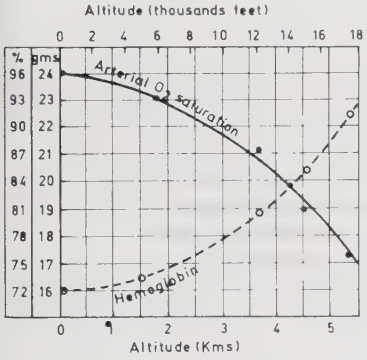

Fig. 1. Hemoglobin concentration (g per 100 ml) and arterial oxygen saturation (%) in healthy male residents at different altitudes (Hurtado et al. (57), courtesy Arch. int. Med.).

Okin *et al.* (86) studied normal hematological standards in adults at two medium high altitudes in the U.S., 1,600 and 3,100 m. When the mean values for hemoglobin in men were plotted in the curve (Fig. 1) there was fairly close agreement. Plotting of other reported mean hemoglobin values from different altitudes (47) shows a wider scatter around the curve, presumably due to differences in material and technique. Walker (119) suggests an increase of hemoglobin concentration of 2.1 % for males and 1.8 % for females for every 1,000 feet elevation. When plotted in the graph, hemoglobin values derived after using this correction factor will, however, lie considerably above the curve. This "standard curve" concerns male subjects.

It is possible by interpolation in the curve to obtain a percent adjustment at a given altitude. Evidence is lacking as to whether such a percent adjustment can be applied to males, females and children alike and whether the same adjustment can also be applied not only to the mean hemoglobin values but also to a lower anemia limit. The range of observations was wider in the higher altitude in the study of Okin *et al.* (86), suggested to be due to increased individual variability of arterial oxygen saturation at higher altitudes. If interpolation is made in the graph (Fig. 1) for the altitudes of Ijaji (1,850 m) and Addis Ababa (2,400 m) and the mean value of 16 g % at sea level is accepted (see Fig. 1) then approximately + 5 % for Ijaji and + 7 % for Addis Ababa should be used as correction factors. This will correspond to roughly 1 g % for adults in Addis Ababa and a little less in Ijaji.

It is obvious that more work is needed to get a definite answer to the question of the influence of medium high altitude on hemoglobin concentration. For the purpose of this study, however, it seemed reasonable to accept the "per cent" adjustments just mentioned, + 5 and + 7 %. These then will be used when comparing mean values found with standard values and when discussing anemia rates.

Results

1. *Adults*

The results of the determinations of Hb, PCV, ESR, SI and TIBC for adult males and females are recorded in *Table 4.* Means and SD for MCHC and transferrin saturation are also entered. In *Figs. 2, 3 and 4* the percentage distributions of Hb, PCV and MCHC are shown.

Although all the subjects were healthy or "apparently healthy" 4 males (4.6 %) and 11 females (22.0 %) had an ESR above 20 mm (maximum 37 mm). 16 of the males (18.4 %) and 34 of the females (68.0 %)

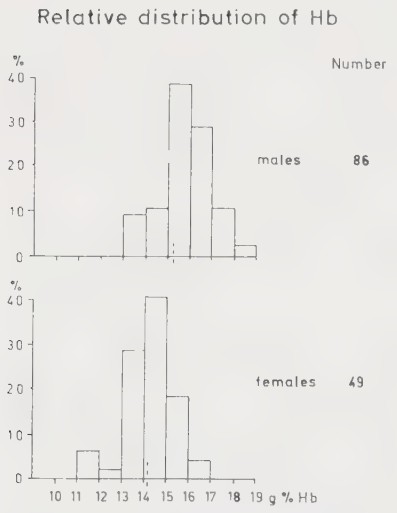

Fig. 2. Relative distribution of Hb in healthy and apparently healthy males and females in Addis Ababa. Interrupted vertical line indicates mean value.

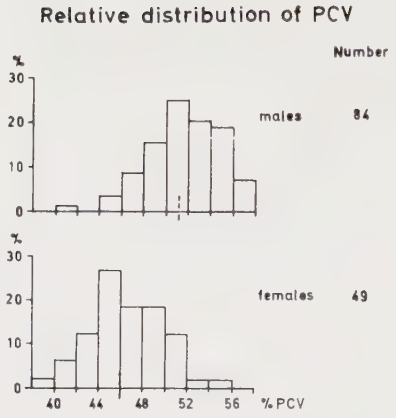

Fig. 3. Relative distribution of PCV in healthy and apparently healthy males and females in Addis Ababa.

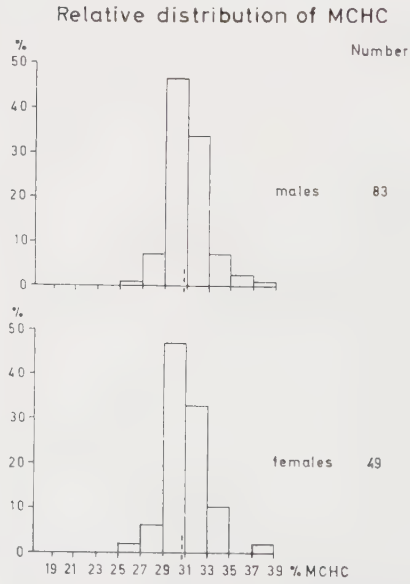

Fig. 4. Relative distribution of MCHC in healthy and apparently healthy males and females in Addis Ababa.

Table 4. *Hematological values in healthy and apparently healthy adult males and females in Addis Ababa.*

		Age	Hb	PCV	MCHC	ESR	SI	TIBC	SI/TIBC
Adult males	Mean	31.4	15.7	51.1	30.9	7.5	125	286	44.1
	S.D.		± 1.11	± 3.16	± 1.72	± 6.39	± 44	± 36	±16.6
		range							
	No.	19—65	86	84	83	87	84	83	83
Adult females*	Mean	23.9	14.2	46.0	30.9	16.0	94	308	31.1
	S.D.		± 1.05	± 3.42	± 2.00	± 7.92	± 41	± 52	±13.6
		range							
	No.	16—45	49	49	49	50	50	50	50

* non-pregnant, non-lactating

Table 5. *Hematological values in "professional" and "non-professional" adults in Addis Ababa. See text for explanation.*

			Hb	PCV	MCHC
Males	"professional"	Mean	16.5	52.7	31.2
		S.D.	± 0.73	± 3.01	± 1.58
		No.	25	25	25
	"non-professional"	Mean	15.4	50.4	30.7
		S.D.	± 1.10	± 2.99	± 1.75
		No.	61	59	58
Females	"professional"	Mean	14.4	45.8	31.4
		S.D.	± 0.88	± 2.99	± 1.74
		No.	28	28	28
	"non-professional"	Mean	14.0	46.4	30.2
		S.D.	± 1.22	± 3.98	±2.26
		No.	21	21	21

had an ESR above 10 mm. The mean Hb values for the subjects with ESR ≤10 mm were, however, very close to those for the whole respective groups — in males 15.9± 1.05 g % (n=70) and in females 14.4±0.96 g % (n=16).

As the material was not homogeneous as regards educational and socioeconomic background an attempt was made to compare the hematological parameters in the "professional" and the "non-professional" groups (cf. "Material"). The result is shown in *Table 5*. In both males and females, the "professional" group had higher mean values for Hb, PCV and MCHC (except for PCV in females). The differences for Hb and PCV between the two male groups were highly significant.* The age range in the "professional" group was 20—35 years and in the "non-professional" 19—65. The mean Hb value in the latter group for the age 20—35 (n=35) was the same as for all "non-professionals".

No "professional" males and 4 "professional" females had an ESR above 20 mm. The mean Hb value for "professional" males with ESR ≤10 mm (22 subjects) and that for "professional" females (13 subjects) were the same as for the whole respective "professional" groups.

In Table 6, the mean Hb values for all adults and for "professionals" only are compared to altitude adjusted mean values given by Wintrobe (123) and Natvig *et al.* (82). For comparison the mean Hb values reported by ICNND (61) for the country as a whole (the majority were residents at an altitude of about or more than 6,000 feet) are also given, as well as those reported by Areskog *et al.* (5) for shoe factory workers in Addis Ababa.

Table 6. *Mean Hb values in adults in the present material compared to "standard values" (adjusted by + 7 % for altitude) and to two previous studies in Ethiopia.*

	Males	Females
Present total material	15.7 (87)*	14.2 (49)
"Professionals" only	16.5 (25)	14.4 (28)
Foreign staff CNU	16.7 (11)	—
Wintrobe** (123)	17.1	15.0
Natvig *et al.*** (82)	16.8	15.3
ICNND country mean (61)	14.4	13.2
Areskog *et al.* (5)	15.6 (42)	—

* number of subjects
** adjusted by + 7 % for altitude

* Throughout this study the following definitions will be used: "Almost significant": P <0.05, "significant": P <0.01, "highly significant": P <0.001.

The mean Hb value for 11 male foreign healthy staff members in Ethio-Swedish projects, who had been resident in Addis Ababa for more than 1/2 year, is also recorded.

As will be seen the mean Hb value for the total Ethiopian males was almost identical with that of shoe factory workers examined by Areskog *et al.* but was more than 1 g % higher than ICNND's mean value for the whole country. It was, however, more than 1 g % lower than the altitude adjusted values given by Wintrobe and Natvig. The mean value in the male "professional" staff and that in foreign staff members agreed well, however, with these standard values.

The total females, although having a higher mean value than the ICNND value for the whole country, were slightly below altitude adjusted standard values. This also held for "professional" females alone, the difference from Natvig's mean value being 0.9 g % and from that of Wintrobe 0.6 g %.

The mean values for SI and TIBC in subjects with ESR ≦10 mm were very close to those for the whole respective groups; in males with a low ESR, the SI value was 129 µg % compared to 125 µg in the whole group, and in females 90 µg % compared to 94 µg % in the whole group.

When only "professionals" with ESR ≦10 mm were included, the mean value for SI still remained at the same level: males 125 µg % and females 92 µg %.

The same was true for mean TIBC values in subjects with a low ESR, although in male "professionals" with a low ESR the mean value was slightly raised from 286 to 304 µg %. This difference is not significant.

Anemia rate

Any discussion about anemia rates will be hampered by the recognized uncertainty of how to define anemia and of the hemoglobin level below which anemia can be said to exist. This problem is even more difficult when altitude adjustment comes into the picture. In clinical work the use of the term marginal zone values is to be preferred to a sharp anemia limit. ICNND, in surveys in Bolivia (60), has differentiated the Hb values as high, acceptable, low and deficient for different altitudes. For the altitude 2,400 m "high" would be above 16.0 g %, "acceptable" 14.5—16.0 g %, "low" 12.5—14.5 g % and "deficient" below approximately 12.5 g %.

For the sake of discussion in this paper it is assumed that the adjustment of +7 % for the Addis Ababa altitude is also applicable to suggested lower hemoglobin limits below which "anemia can be said to exist" although it is recognized that such an assumption may be debatable.

In *Table 7* the percentage of anemic adult subjects is given, using 3 different altitude adjusted standards.

Table 7. *Percentage of anemia in adults in Addis Ababa according to 3 different criteria, with adjustment for altitude.*

	WHO* (62)	Wintrobe** (123)	Natvig *et al.**** (82)
Males	7.0	19.8	19.8
Females	8.2	8.2	16.3

* males < 13.9 g %
females < 12.8 g %
(see text)

** males < 15.0 g %
females < 12.8 g %

*** males < 15.0 g %
females < 13.4 g %

It is obvious that with different comparison standards the percentages of anemia in the present material will vary a great deal. This would be the case even if the standards were not altitude adjusted. The large differences in anemia rates when different standards are applied also indicate that a number of cases are marginally low.

The range of Hb values in the present material for males was 13.2—18.6 g % and for females 11.6—16.3 g %. 6 males and 4 females had Hb values below the anemia limit recommended by WHO (62), adjusted

for altitude, viz. 13.9 g % for males and 12.8 g % for females.*

Two of the 6 males had a moderately raised ESR (24 and 29 mm). In 1 case there was no record of PCV but the remaining 5 had a PCV below the mean value for the whole group although all were within the mean — 2 SD. In all these 5, MCHC was below the mean value for the whole group but in only 1 of them was it below the mean — 2 SD. In the latter subject SI was 119 and TIBC 247 µg %. Finally in all 6, SI was below the mean value for the group. It should be pointed out that all the 6 "anemic" males had stainable hemosiderin in the bone marrow and that all belonged to the "non-professional" group. It should also be noted that 4 of the 6 males were above 45 years of age. In the total material only 14 % exceeded 45 years.

Two of the 4 females had a moderately raised ESR (33 and 35 mm). In all the 4 the PCV and MCHC values were below the mean for the whole group. One had a PCV value below the mean — 2 SD for the whole group and another had MCHC below the mean — 2 SD. The last mentioned had delivered 8 months earlier. Her SI value was, however, 80 µg % combined with a low TIBC, and therefore did not present a picture typical for iron deficiency anemia. Two had SI values below and 2 above the mean for the whole group. One anemic female was "professional".

In 5 females and no males SI was below 50 µg %, slightly lower than the normal limit given by Wintrobe (123). None were anemic according to previously discussed criteria but all had an ESR above 10 mm although only 2 had definitely pathological values (34 and 37 mm) suggestive of recent or present infection.

* WHO recommended in 1959 (62) a lower limit of 14.0 g % for males, 12.0 g % for non-pregnant females and 10.0 g % for pregnant females. These limits have been revised in 1968 and the new recommended anemia limits will be 13.0, 12.0 and 11.0 g %, respectively (Professor L. Hallberg, personal communication). They will be used throughout this paper.

Comments

Areskog *et al.* (5), on examining Air Force cadets in Debre Zeit, outside Addis Ababa, at an altitude of about 1,900 m, found in this homogeneous group of physically fit young men a mean Hb of 15.9±0.9 g %. A minor upward correction of about 0.2—0.3 g %, as according to the above discussion on this topic, would make a comparison to the Addis Ababa altitude possible. In the same study (5) the mean Hb in shoe factory workers in Addis Ababa was found to be 15.6±1.2 g %.

The present series, although heterogeneous from an educational and socio-economic point of view, was considered medically fit, and, although no special dietary data were obtained, all subjects were known to consume the traditional Ethiopian diet, meaning a high dietary iron intake. The intake of other nutrients necessary for hematopoiesis was not specifically studied, although the protein intake can be assumed to correspond to, or even, especially in the "professional" groups, exceed that of the shoe factory workers in the study of Areskog *et al.* (5), at least as regards the intake of animal protein.

The mean Hb value of 15.7 g % in males in the present material agrees well with those in the two materials of Areskog *et al.*, although there is a difference of slightly more than 1 g % from altitude adjusted "standard values". In the selected group of "professionals", however, the mean Hb value of 16.5 g % came close to the reference standard used and would be in agreement with other standards, for instance that proposed by Vahlquist (116) (16.5 g % when adjusted by +7 % for altitude).

The difference between the mean Hb in males and females in most "standard materials" is usually about 1.5—2 g %. This is true also for the present material, where the difference is 1.5 g %, increasing to 2.1 g % if "professional" males and females are compared. The difference between the mean Hb for all females in the present material and the altitude adjusted mean values of the reference

standards used (Table 6) is 0.8—1.1 g %, but only 0.5 g % when comparison is made with the altitude adjusted mean value of 14.7 g % proposed by Vahlquist (116).

In view of the fact that agreement between different "standard values" published is by no means complete due to differences in material and techniques, and the rather imprecise way of choosing the factor for altitude adjustment, the mean values obtained in the selected groups of young and medically fit "professionals" in the present material can be considered reasonably comparable to other standard values reported.

The cases of anemia in males diagnosed according to the criteria of WHO were apparently not due to iron deficiency, as all had stainable hemosiderin in the bone marrow, most of them in abundance. The fact that 4 of the 6 males diagnosed as anemic belonged to the oldest in the material (49 years or more) cannot be satisfactorily explained. There was a successive decrease in mean Hb from 16.0 g % in subjects below 25 years to 15.1 g % in ages ≧45 although this may be explained by the fact that the younger groups included the "professional" staff. ICNND (61), however, reports the same decline in mean Hb with increasing age.

The state of the iron stores in adult healthy Ethiopian females is not known although it is reported from South Africa (10) that Bantu females with a high iron intake had much less siderosis than males and that about 75 % had iron concentrations in the liver which were normal or very close to normal. The 4 females in this material did not present any picture typical of iron deficiency. All females except 1 were below 45 years, so that an age group comparison with the males was not possible.

The mean value of 125 µg % for SI in males is in satisfactory agreement with that for other workers, while the value for females, 94 µg %, is slightly lower than most reported mean values (8). In both males and females, the mean values for TIBC were slightly lower than those mostly reported (8).

There are no indications that chronic exposure to high altitudes significantly affects SI or TIBC (95, 112).

2. "Privileged" children 1—14 years

The results of determination of Hb, PCV, ESR, SI and TIBC as well as the results of MCHC and transferrin saturation for the different age groups are given in Table 8. The sexes are not treated separately. *Figs. 5, 6 and 7* show the percentage distributions of Hb, PCV and MCHC.

Although, as mentioned previously, all the children were in good health and none showed any signs of relevant skin infection or other infectious ailments at the clinical examination, a considerable number had a raised ESR. In the age group 1—4 years 28.1 % had an ESR

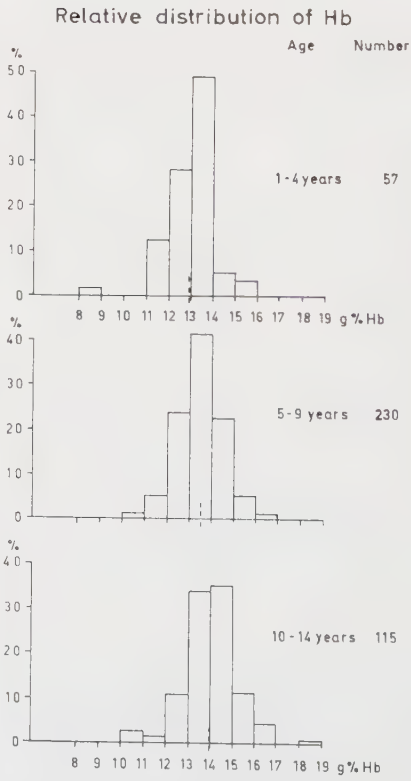

Fig. 5. Relative distribution of Hb in "privileged children" 1—14 years in Addis Ababa.

higher than 20 mm (maximum 39 mm), in the group 5—9 years 15.4 % (maximum 40 mm) and in the group 10—14 years 12.3 % (maximum 36 mm). The falling rate is in agreement with the decreasing mean ESR with increasing age (cf. Table 8). The mean Hb value for the children with ESR ≦20 mm was, however, very close to that for the whole respective group — in the age groups 1—4 and 10—14 years it was only 0.1 g %

Table 8. *Hematological values in "privileged children" 1—14 years* in Addis Ababa.*

		Hb	PCV	MCHC	ESR	SI	TIBC	SI/TIBC
1— 4 years	Mean	12.9	42.0	30.8	17.5	89	323	28.9
	S.D.	± 1.09	± 2.43	± 1.97	± 7.47	±30	± 50	± 9.5
	No.	57	51	51	57	26	26	26
5— 9 years	Mean	13.5	43.8	30.8	13.3			
	S.D.	± 0.97	± 2.98	± 2.09	± 7.45			
	No.	230	222	222	227	93	305	30.5
						±35	± 44	±10.0
						82	82	82
10—14 years	Mean	13.9	44.8	31.1	12.7			
	S.D.	± 1.18	± 2.54	± 2.20	± 6.95			
	No.	115	114	114	114			

* both sexes

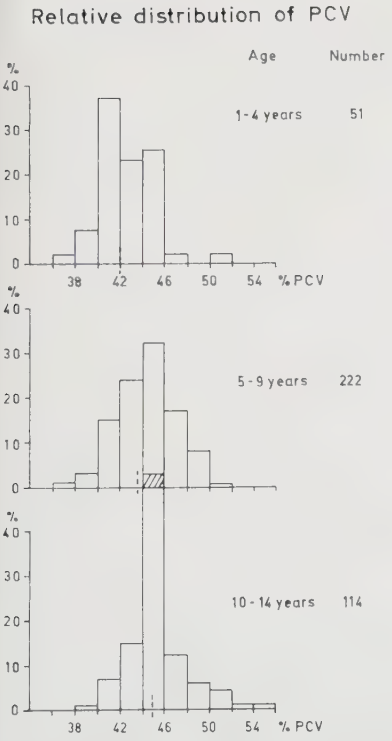

Fig. 6. Relative distribution of PCV in "privileged children" 1—14 years in Addis Ababa.

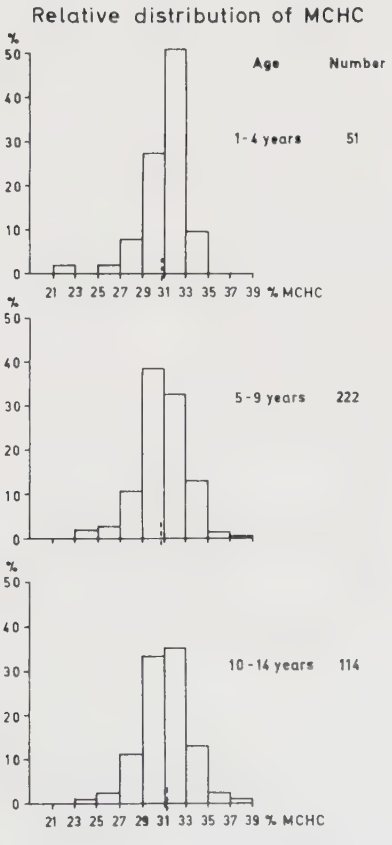

Fig. 7. Relative distribution of MCHC in "privileged children" 1—14 years in Addis Ababa.

higher while it was exactly the same in the 5—9 year age group. When, however, only children with ESR ≤10 mm were included, the mean Hb values for the three age groups increased slightly and were almost exactly the same as the altitude adjusted values given by Wintrobe (123) and Natvig *et al.* (83) (see *Table 9*). It appears that in the age group 1—4 years 78.9 % had an ESR above 10 mm, in the group 5—9 years this value was 59.6 % and in the group 10—14 years 54.8 %.

The mean values for PCV in children with ESR ≤10 mm were also slightly higher, making the mean MCHC value remain at the same level.

The means for SI and TIBC in children with ESR ≤10 mm in the 5—14 year age group were 98 and 306 µg %, respectively, (n=37), thus close to the mean values for the whole group. In the group 1—4 years, SI and TIBC were recorded in only 5 children with ESR ≤10 mm.

Anemia rate

As with the adults the same altitude adjustment (+7%) will be applied to some suggested lower hemoglobin levels below which "anemia can be said to exist". In *Table 10* the anemia limits suggested by WHO (62) and Natvig *et al.* (83) are used as references. WHO proposes for the age group ½—4 years 10.8 g %, for 5—9 years 11.5 g % and for 10—14 years 12.5 g %. Natvig *et al.* gives for children 7—9 years a lower limit of 11.1 g % and for 10—13 years 11.6 g %.

Table 10. *Percentage of anemia in "privileged children" 1—14 years according to criteria (altitude adjusted) of WHO* (62) and Natvig et al.** (83).*

	WHO (62)	Natvig *et al.* (83)
1— 4 years	5.3	—
5— 9 years	7.8	5.7
10—14 years	30.4	7.0

* ½— 4 years: Hb <11.6 g %
5— 9 years: Hb <12.3 g %
10—14 years: Hb <13.4 g %

** 7— 9 years: Hb <11.9 g %
10—13 years: Hb <12.4 g %

In view of the fact that Natvig's proposed "normal values" are derived from a very carefully checked and iron supplemented series it would seem probable that the "anemia limit" suggested by WHO for children 10—14 years is too high. This is also supported by the normal ranges given by Vahlquist (116) and Wintrobe (123).

Table 9. *Hematological values in children with ESR ≤10 mm, compared to altitude adjusted "standard values" for Hb.*

		Hb	PCV	MCHC	Wintrobe* (123)	Natvig *et al.** (83)
1— 4 years	Mean	13.4	43.5	30.8	13.4	—
	S.D.	± 0.83	± 3.06	± 1.33		
	No.	12	10	10		
5— 9 years	Mean	13.6	44.4	30.6	13.8	13.6**
	S.D.	± 1.16	± 2.60	± 2.47		
	No.	93	89	89		
10—14 years	Mean	14.3	45.2	31.6	14.3	14.1***
	S.D.	± 1.17	± 2.66	± 2.01		
	No.	52	52	52		

* adjusted for altitude by + 7 %
** 7— 9 years
*** 10—13 years

In an analysis of the children who were anemic according to the criteria of WHO, the following was found.

1—4 years. 3 out of 57 were anemic according to WHO (Hb range 8.1—11.3 g %). One child, 1 year old, had a marked hypochromic anemia (Hb 8.1 g % and MCHC 22.5 %). The other 2 had marginally low Hb values of 11.1—11.3 g %. MCHC recorded in 1 of those, was 27.8 %. The ESR values ranged between 18—28 mm.

5—9 years. 18 out of 230 were anemic according to WHO (Hb range 10.3—12.2 g %). In 8 of them MCHC was below 27 % (9 in the whole age group). In 3 of the 18 the ESR values were above 20 mm or about the same percentage as for the whole age group.

10—14 years. 35 out of 115 were anemic according to WHO (Hb range 10.4—13.3 g %). In 4 of these 35, MCHC was below 27 %. In 3 of them the ESR value was above 20 mm — or about the same percentage as in the whole age group. 5 children (4.3 %) had a Hb value below 12 g % and 17 (14.8 %) below 13 g %, indicating that the majority of the anemic cases were marginally low. Only 8 children were anemic according to the altitude adjusted criteria of Natvig. In 4 of these, MCHC was below 27 %.

In 8 of 108 children the SI value was below 50 µg % (range 30—49). None of these 8 had an ESR above 20 mm or were anemic according to any criteria discussed.

Comments

The mean Hb values especially in children with ESR ≦10 mm were in good agreement with the altitude adjusted "standard values" given by Wintrobe (123) and Natvig *et al.* (83). The values for the three age groups were also in agreement with altitude adjusted values proposed by Vahlquist (116) for the ages 3, 7 and 11 years, respectively.

Further, they agreed with the means given by Areskog *et al.* (5) for children tested for physical fitness at the French school in Addis Ababa. ICNND (61), using a different age group system, found for school age boys ≦9 years a mean Hb of 12.5 g % and for school age boys below 15 years 13.7 g % (the girls had nearly the same mean values), which seemingly is a little lower than in the present study. The mean MCHC for these groups were the same as in this study.

The mean values for SI in ages 1—14 years (Table 8) were slightly lower than means reported by Hagberg (40) but agree better with those of Vahlquist (115). The mean values for TIBC were also lower than those reported by Hagberg (40).

The total anemia rate is difficult to evaluate in view of the considerable difference in the age group 10—14 years between the two criteria of WHO (62) and Natvig *et al.* (83). As already indicated it would seem that the anemia limit suggested by WHO in this age group is a little too high. If that of Natvig is applied for this age group and those of WHO for ages 1—9 years, the total anemia rate would be 7.2 %.

MCHC has been claimed to be an insensitive parameter for diagnosing iron deficiency anemia (32). In adults and in children the mean MCHC in this study was about 31 % with 2 SD about 4, which also corresponds to the variation given by Natvig *et al.* (83) in subjects 7—20 years old. Throughout this study an MCHC value of below 27 % will be used to denote hypochromia, in anemic subjects suggestive of iron deficiency anemia, although the anemia of chronic infection may sometimes be hypochromic (123). In view of findings of Garby *et al.* (32) it is possible that the rate of iron deficiency anemia may be underestimated, especially in situations where iron deficiency is not uncommon.

According to this criteria and the anemia criteria of WHO (62) for the age groups 1—9 years and those of Natvig *et al.* (83) for 10—14 years, 3.4 % of the children had an iron deficiency anemia.

Dietary data (101) indicate an increasing iron intake once the children start to participate in the adult iron rich diet. In the French school in the age group 9—14 years a daily average iron intake of about 300 mg was found (5).

No stool tests were made in these children in the present study and although hookworm is not seen in Addis Ababa a few children with suggestive iron deficiency anemia may have brought an infestation from the rural areas. The one year old child referred to previously, most certainly had developed anemia due to an iron deficient monotonous milk diet.

The high mean values for ESR and the high rate of children with an ESR of more than 20 mm should be noted in view of the fact that the clinical examination did not reveal any signs of infections. However, in the surveys of ICNND (61) and CNU (77) high levels of gamma-globulins have been found already from early ages. ICNND (61) reported that the gamma-globulin was elevated in all but the breastfed infants. These findings are suggestive of early contraction of significant but often subclinical infections. The levels of the immunoglobulins G and D have been found highly significantly raised above those found in Swedish children of the same age (66).

3. Pregnant women and mothers at delivery

The results of the determinations of Hb, PCV, SI and TIBC as well as MCHC and transferrin saturation are given in Table 11. The hematological parameters of the mothers at delivery are also recorded and for comparison those of non-pregnant, apparently healthy females.

The range of Hb in the expectant women was 10.7—15.8 g % and in the mothers at delivery 11.1—16.3 g %. A total of 5 women or 6.6 % would be classified as anemic if the new WHO recommendations (see footnote page 25 were followed — a lower limit of

Table 11. *Hematological values in pregnant women and mothers at delivery (compared to non-pregnant females) in Addis Ababa.*

		Age	Hb	PCV	MCHC	SI	TIBC	% satur.
7th month	Mean	26.4	12.6	40.9	31.0	91	370	25.4
	S.D.		± 1.06	± 4.34	± 2.88	± 34	± 50	±11.1
	No.	18—36*	9	9	9	9	9	9
8th month	Mean	24.3	13.1	42.3	30.7	104	417	25.6
	S.D.		± 1.11	± 3.91	± 2.24	± 24	± 60	± 7.6
	No.	17—39	22	22	22	21	21	21
9th month	Mean	25.6	13.4	44.2	30.4	137	402	34.3
	S.D.		± 0.88	± 3.41	± 2.53	± 53	± 40	±13.7
	No.	16—37	21	21	21	20	20	20
7—9th month	Mean	25.2	13.1	42.8	30.6	115	402	29.1
	S.D.		± 1.03	± 3.92	± 2.43	± 43	± 53	±11.6
	No.	16—39	52	52	52	50	50	50
At delivery	Mean	26.0	13.8	45.1	30.6	82	380	21.3
	S.D.		± 1.20	± 2.84	± 2.02	± 42	± 62	±10.0
	No.	16—43	24	24	24	26	26	26
Non-pregnant females 50**	Mean	23.9	14.2	46.0	30.9	94	308	31.1
	S.D.		± 1.05	± 3.42	± 2.00	± 41	± 52	±13.6

* age range
** number. See Table 4.

11.0 g % adjusted by +7 % for altitude = 11.8 g %. One was delivering and 4 were in the 7—8th month of gestation. Their Hb values were marginally low, 10.7—11.7 g %, and all had MCHC values above 27 %. Their SI ranged between 61 and 106 µg %.

As can be seen in Table 11 there was a gradual increase in the mean Hb and PCV values from the 7th month of gestation up to the time of delivery, while the mean MCHC remained at about the same level. At delivery the mean Hb, PCV and MCHC values were close to those in non-pregnant females. The differences in Hb and PCV between the 7th and 9th months were almost significant. The mean SI value also showed a gradual increase up to the 9th month, when in fact it exceeded that of non-pregnant females (highly significant difference). The mean value at delivery is not fully comparable as blood from these women was drawn at any time of the day, while in the expectant mothers it was taken only at 9—11 a.m. The mean TIBC values remained throughout, with moderate fluctuations, on a markedly higher level than those in non-pregnant females. The % transferrin saturation at the 9th month slightly exceeded that in non-pregnant females. When the expectant women (7—9th month) were divided according to *parity* (1st pregnancy (n=10), 2nd—3rd pregnancy (n=15) and ≧4th pregnancy (n=27)) the mean Hb values showed no consistent trend — for the three groups they were 13.3, 12.6 and 13.4 g %, respectively. The mean values for SI, on the other hand, showed an upward trend — 92, 103 and 129 µg % for the three groups, respectively. The difference between the lowest and the highest values is almost significant .The mean ages in these three groups were 21.9, 21.8 and 28.3 years, respectively.

When the expectant women were divided according to *age* groups of 15—20 years (n=9), 20—29 years (n=30) and 30 years or more (n=13) the mean Hb values still showed no consistent trend — 13.4, 13.0 and 13.2 g %, respectively. The mean values for SI, on the other hand, showed an upward trend — for the 3 groups these were 96, 117 and 124 µg %, respectively. The difference between the lowest and highest values is not significant.

The same was found when the women were divided into only two age groups, 15—24 years (n=25) and ≧25 years (n=27). Here the mean Hb values were 13.2 and 13.1 g % and the mean SI values 101 and 127 µg %, respectively. The difference is almost significant.

Comments

In normal pregnancy the total blood volume increases by about 35 %. Most of this is due to an increment in the plasma volume, with only a modest increase in the red cell volume. The plasma volume increases up to about the 34th week, after which there is a decline, while there seems to be a linear increase in the red cell volume up to term (59).

In the peripheral blood, the hemoglobin concentration usually falls, starting already during the first trimester, parallelled by a fall in the hematocrit and red cell count (59). The minimum is reached at about the 30th —32nd weeks or a little later, followed by a slight rise at the end of pregnancy (59). MCHC appears to vary little during pregnancy, although in some series (59, 109) a slight fall has been reported. Sturgeon (109) found a fall from 31.6 % in early pregnancy to 30.9 % in late pregnancy in women who received no iron, while iron treated groups showed no such reduction. Many workers have reported (see Hytten *et al.* (59)) that the mean hemoglobin concentration in women given therapeutic doses of iron by mouth or parenterally rose during pregnancy, sometimes to non-pregnant levels, and that the usual fall in hematocrit could be similarly modified by iron. Gerritsen and Walker (33) have reported that in Bantu women with an exceptionally high habitual dietary intake no

fall in mean hemoglobin concentration or mean hematocrit occurred during pregnancy.

The average serum iron concentration is usually reduced to about 35 % below the mean for non-pregnant women and the transferrin level raised, resulting in a low percentage saturation (59), making the picture resemble that in iron deficiency anemia. Iron medication can arrest or modify this fall in serum iron (109). In the Bantu women there was no reduction at all, although the transferrin levels increased (33).

In the present material there was a difference of 1.6 g % between the mean Hb value in the 7th month of gestation and that of non-pregnant females. What is interesting is that it successively increased up to parturition, coming very close to the non-pregnant mean value and being parallelled by a corresponding rise in the mean PCV value. The mean Hb value for all pregnant females including those at delivery was 13.3 g %. This is less than 1 g % lower than that for non-pregnant females and although this difference is greater than that reported from South Africa in Bantu females (33), it is smaller than in most reported series without iron treatment (59). ICNND (61) found in Ethiopian pregnant women at the same altitude as the present investigation, a mean Hb of 14.0 g % in the first trimester and of 13.1 g % in the third, or the same as in this study, and also the same mean PCV and MCHC. Only 2 of the 96 examined pregnant females in their series showed evidence suggestive of iron deficiency anemia.

Also the mean value for SI increased from the 7th month and onwards and almost significantly exceeded that of non-pregnant females in the 9th month. The median value in this group, 116 µg %, is, however, probably more representative than the mean value of 137 µg %, which may be distorted by two very high values, well above 200 µg %. The same also applies to the high mean values found in those pregnant for the 4th or more times (mean value 129 µg %, median value 116 µg %) and those ≧25 years (mean value 127 µg %, median value 114 µg %). The almost significiant difference between these means and those in the lowest parity group and age group, respectively, is therefore questionable. The mean value for SI for all expectant women, 115 µg % (median 111 µg %) is in good agreement with mean values reported for non-pregnant females (40, 115) and is slightly, although not significantly, higher than the mean value in non-pregnant Ethiopian females. In any case, the usually recorded decrease in SI of about 35 % compared to non-pregnant values was not met here and the findings are in agreement with the value reported for pregnant Bantu females in the last trimester, 129 µg % (33).

The increase in the TIBC levels in the present material also corresponds to that in pregnant Bantu women, where the mean value in the last trimester was 403 µg %, compared with 323 µg % in non-pregnant females (33). In the present material the corresponding values were 402 and 308 µg %, respectively.

The similarity to the findings in pregnant Bantu women who also have a high dietary iron intake is pronounced.

There is evidence that iron absorption during pregnancy is increased severalfold. Hahn *et al.* (42) found that from the 15th week of pregnancy the iron absorption started to increase and after week 34 it was nearly 4 times that of weeks 10—14. Hallberg and Rybo (97) found an increased absorption of between 2 and 17 times. Their subjects had first received iron injections to exclude the possibility that an iron deficiency would promote improved absorption. They concluded that it is not the availability of iron in the body *per se* which is responsible for the increased iron absorption but rather that the iron needs of the fetus, placenta and bone marrow are so high that the mobilization from the stores cannot keep pace.

It seems conceivable, therefore, that in the present material the pregnant females not

given iron medication but having a habitually high dietary intake of iron would absorb more than those in the non-pregnant state and that this additional iron absorbed — as in iron medication — would in the first place prevent iron deficiency anemia and secondly would result in only a slight or moderate fall of the hemoglobin concentration, also arresting the usual fall in serum iron concentration — all laboratory signs which have been ascribed to iron deficiency (41, 109). The possibility that increased mobilization from presumed large iron stores occurs during pregnancy must also be kept in mind, although in non-pregnant Bantu females the stores were essentially within the normal range (10).

4. Newborn infants

The results of the determinations of Hb, PCV, SI and TIBC as well as MCHC and transferrin saturation are given in Table 12. It should be noted (cf. "Material") that determinations of Hb and PCV were made on capillary blood and SI and TIBC on cord blood (on other infants).

As is evident from the high figures of standard variations from the means of Hb and PCV the individual values were widely scattered, although the mean MCHC agreed well with that of all other age groups. The range of Hb values was 12.3—28.9 g % and that for PCV 41—85 %. The two extreme Hb and PCV values were obtained from the same two children. One forceps delivered girl with a birth weight of 2,700 g was most certainly anemic (Hb 12.3 g %, PCV 41 % and MCHC 30.0 %).

The high SI and low TIBC should be compared to the corresponding values in mothers at delivery who in this material, as in others previously referred to, had considerably lower SI and higher TIBC values.

Comments

It is difficult to compare the mean values for Hb and PCV with "standard values", as the relative intra-uterine hypoxia in the mothers, who are already affected by the relative hypoxia at the altitude of 2,400 m, may justify a higher correction factor than +7 % (cf. Fig. 1). In addition, "normal values" reported for capillary blood at birth (1st day) vary a great deal. It has also been shown (85) that in infants with the cord clamped late there is a temporary rise in the capillary PCV value, with a maximum at 6 hours after birth followed by a fall in the next 6 hours back to approximately initial values, which persist for the next 2—3 days. In the study of Oh and Lind (85) it was also shown that the capillary hematocrit values were higher when taken from pin-pricks in warmed than in unwarmed heels. The majority of the infants in this material were tested between 6 and 24 hours after birth and always by pin-pricks in unwarmed finger tips. In the obstetric routine at the hospital where the tests were made the cord is not clamped until all pulsations have ceased.

Vahlquist (115) found in capillary blood on the first day a mean Hb value of 18.4 ± 2.62 g %, Sturgeon (110) gives 18.1 ± 2.3 and

Table 12. *Hematological values in newborn infants in Addis Ababa.*

	Hb*	PCV*	MCHC	SI**	TIBC**	% satur.
Mean	21.0	68.3	30.9	150	267	57.3
S.D.	± 3.14	±10.37	± 2.90	± 55	± 59	±19.3
No.	30	25	25	47	44	44

* Capillary blood at <36 hours' age
** Cord blood

Moe (79) a value of 19.8±2.4 g % (2nd—6th day). Albritton (2) reported higher values and gave for the 1st day a value of 21.2 g % with a range of 17.7—26.5 g %.

The mean Hb value given by Wintrobe (123), which was compiled from several sources, adjusted by +7 % for altitude, will be 20.9 g %, which compares favourably with the mean value in the present material, although as was pointed out, the altitude correction factor should possibly be higher. The PCV value is even more difficult to compare with standard values due to probable differences in technique.

The mean value for SI agrees with the findings of Vahlquist (115) and Laurell (67) but is lower than that reported by, for instance, Hagberg (40) and Sturgeon (110). The marked difference in TIBC between the maternal and the cord blood is in good agreement with the findings of others (40, 67, 110) as well as the great difference in transferrin saturation.

Sturgeon (109, 110), in a carefully controlled material of pregnant mothers, one group of whom was given no iron and two other groups iron supplemented, found no difference in mean Hb and SI in the offspring in the three groups at birth and up to 18 months. With respect to the high dietary iron intake in the mothers, the hematological picture in the newborn as shown in this material displays no particular signs which could be attributed to excessive maternal iron load.

Summary

A total of 615 healthy and apparently healthy subjects aged 1—65 years were examined hematologically. In addition the capillary blood was examined in 30 newborn infants and the cord blood in another 47.

In general the mean hemoglobin values in the different age and sex groups agreed well with accepted "standard values", adjusted for altitude by +7 %. In adult males there was a significant difference in mean Hb between "professionals" and "non-professionals", only the former group being comparable to "standard values" and to the mean value found in foreign residents. In adult females the corresponding difference was smaller and non-significant.

In children aged 1—14 years there was good agreement with "standard values" especially in children with a low ESR. There was also a satisfactory agreement in newborn children.

In pregnant and delivering mothers there was only a comparatively small decrease in mean Hb values compared to non-pregnant females.

The mean MCHC was approximately 31 % in all groups.

The mean serum iron level remained constant from before 5 years and upwards, with an increase to higher values after puberty in males, which is a pattern observed in other investigations, while TIBC rather exhibited the opposite pattern with higher values in early childhood and lower values especially in adult males, also confirming the findings in other studies.

Contrary to what is usually observed, the serum iron in the pregnant females in the 3rd trimester was not decreased compared to non-pregnant females.

The anemia rate, according to the criteria discussed, was low, approximately 7—8 %. No definite cases of iron deficiency anemia were diagnosed in adults — identified as anemia with MCHC below 27 % — while in children 1—14 years about 3 % appeared to have this type of anemia.

It would therefore be justified to conclude that the iron nutrition on the whole seemed to be adequate in the subjects under study.

CHAPTER IV.

Evaluation of the iron stores

Introduction. Oral iron overload

The body of the normal adult male contains about 3—4 g iron (8). Approximately 65 % of this iron is contained in the circulating hemoglobin, some 30 % is storage iron and the remainder is to be found in myoglobin, the heme enzymes and in the plasma (8).

After adulthood is reached, about 1 g storage iron is present in the body (8). This store remains essentially unchanged in males during the remainder of life since the amounts of iron absorbed are normally sufficient to balance the small amounts excreted. In women, menstruation and pregnancy may cause a drain and even depletion of the stores. An absolute increase in the iron content of the body means that more iron has been absorbed than excreted or that iron has been introduced via some other route. In both instances the excess iron is laid down in stores in the bone marrow, liver and spleen as ferritin and hemosiderin (8).

The average healthy adult male in temperate climates loses normally about 0.6 mg of iron daily in the stools, urine, sweat, hair and desquamated cells (8). A female loses approximately double that amount during the reproductive period (8), however, with considerable individual variations (84).

The absorption, in contrast to the excretion, may vary within a wide range. Iron in the divalent, ferrous form is more easily absorbed than in the trivalent, ferric form (14). The absorption is favoured by large doses of ascorbic acid (15), mainly due to its reducing action in the intestines, while phytates, phosphates and excess calcium may have an unfavourable effect on the absorption of iron although their importance in the normal diet is uncertain (81). The role of the gastric juices in iron absorption is subject to conflicting views but it appears that pancreatic secretions may have an inhibitory effect (8).

The presence of clay in the food (geophagia) has been shown to diminish absorption (78). There are good reasons to believe that general bulkiness of the food, as is common in developing countries, will have an unfavourable effect on iron absorption. However, surprisingly few facts are available in this field.

The amount of iron in the tissue stores is also of importance. A decrease results in enhanced absorption and *vice versa* (11, 91). There is increased absorption when erythropoiesis is stimulated by bleeding, by hemolytic agents, by cobalt (11) and by the relative anoxia of high altitudes (95), while the opposite occurs when erythropoiesis is depressed (11, 95).

A gross increase in the body iron can arise from a habitually high dietary iron intake as among the Bantu (7, 21, 22, 38, 53, 118) or from habitual iron medication over several decades (114). In idiopathic hemochromatosis the excessive iron deposits have been ascribed to an increased absorption from a normal diet due to a presumed metabolic error (8), although this view has been questioned (71, 72). It is also seen in certain anemias and as a result of frequent transfusions (for comprehensive reviews see refs. 8 and 71). An excessive dietary iron intake has been reported to occur primarily among the Bantu population in Southern Africa (120) and in Ethiopia (61). From some other parts of the world, a high iron intake has been connected with consumption of iron rich wines (71) or drinking water (55).

The high intake of iron among the Bantu, amounting to 100 mg daily or more, is derived mainly from iron utensils used in cooking and in the preparation of kaffir beer (120). It has been calculated that a daily retention in the male of only 2.5 mg iron between the ages 20 and 40 years would lead to an accumulation of about 10 g iron by the age of 40 years (7). Other factors may, however, be of importance in producing the hemosiderosis which is commonly seen in the male Bantu, especially after the age of 40 years. It has been suggested that the increased absorption might be a result of a widespread metabolic defect induced by chronic malnutrition (36, 39). However, the degree of siderosis is reported to be the same in apparently well-nourished subjects (7, 53). Poor diet in animal experiments together with a low phosphorus content can, however, result in increased absorption of iron and massive iron storage (37). Even in animals on grossly deficient diets, an iron overload can largely be prevented by the addition of phosphates (51), suggesting that the excessive absorption in the animals is related more to the iron/phosphorus ratio of the diet than to an alteration of the intestinal mucosa caused by malnutrition. The overall phosphorus content in the average Bantu diet is, however, if anything greater than normal (120).

It has also been considered feasible that a variety of unidentified toxic substances in alcoholic concoctions consumed by the Bantu in large amounts could, together with malnutrition and hepatotoxic viruses, be responsible for potentiation of the liver damaging effects of large liver iron deposits (54).

The incidence of hemosiderosis in the Bantu, identified as chemically determined liver iron exceeding 0.1 % of dry weight, has been reported to be 89 % in traumatic deaths (7). The incidence and severity tend to be distinctly lower in females than in males (10). The siderosis first becomes manifest in late adolescence and reaches its greatest severity after the age of 40 years. The possible relationship between iron overload and tissue damage is subject to conflicting views (19, 71, 72). Some investigators have found cirrhosis of the liver in Bantu subjects with all grades of siderosis (39), while others (38, 54, 63) have found a close relationship between hemosiderosis and the more severe degrees of portal fibrosis or cirrhosis. A combined chemical and histological study of 147 livers from persons who died accidentally showed portal fibrosis or cirrhosis in all cases where the iron concentration was above 2 % of dry weight (7). In another study (63) severe hepatic siderosis was present in 22 of 24 patients with cirrhosis; the average liver iron concentration for the whole group being 2.3 % of dry weight. In the cirrhotic cases there were significant epithelial deposits in other organs making the overall histological picture virtually indistinguishable from idiopathic hemochromatosis. Parenchymal deposits in other organs do not seem to occur unless cirrhosis is present (63), causing a widespread redistribution of iron. Seven per cent of a randomly selected series of 100 diabetic Bantu subjects were diagnosed as having fully developed hemochromatosis, an incidence more than 40 times greater than that of an American series of diabetic subjects (100). The only finding which distinguished the Bantu hemochromatosis from the idiopathic type was the high iron concentration present also in the spleen (63).

Available evidence seems to indicate that the high iron intake *per se* is mainly responsible for the iron overload in the Bantu and that when excessive parenchymal deposits of iron are present for a long period, characteristic pathological and clinical features may develop. Other factors such as malnutrition, alcoholism, toxins and viral hepatitis may predispose to, or potentiate the effects.

A. Studies on bone marrow hemosiderin in relation to certain other hematological parameters and age

The most direct and practical method of assessing the iron stores is examination of fragments of bone marrow for stainable hemosiderin (8). The method appears to be a valid one since comparable results have been obtained when the non-hemoglobin iron in marrow aspirates has been estimated chemically (30, 121). Weinfeld (121) found a highly significant correlation between the two methods. For each histochemical group the mean of the chemical determination was significantly different from that of either of adjacent groups. There was, however, considerable overlapping, especially in the groups with higher hemosiderin scores. He found no difference between smear technique and that of histological sections as regards the means of chemically determined non-hemin iron for the respective histochemical grades. He also found a highly significant correlation between the iron stores in the liver and the bone marrow in basal subjects, although the scatter of individual values was appreciable.

It would thus seem that the bone marrow hemosiderin smear method should be satisfactory for screening purposes in the estimation of the reticuloendothelial iron stores, although it should be emphasized that this method gives no information on the ferritin part of the stores.

The object of the present investigation was to study the bone marrow hemosiderin in relation to age and to certain other hematological parameters.

Material and methods.

Bone marrow aspirates were taken from 149 subjects of ages 3—65 years. 13 were excluded — 7 because of technical failure to obtain enough marrow and 6 when found to have leprosy under treatment. 136 healthy and apparently healthy subjects thus remained. The children under 10 years (of both sexes) belonged to the Children's Home which is run by CNU. The teenage boys, who volunteered, were inmates at the Home for Delinquent Boys* in Addis Ababa where CNU had made examinations during the course of a supplementary feeding program. The adult men, also volunteers, were staff members of CNU and ESPC or maintenance workers at the ammunition factory in Addis Ababa*. A few samples were also taken from patients who had recovered from minor illnesses at the Body Guard Hospital.**

All were given a brief physical examination to rule out frank infections and other disorders. From all except 5, venous blood was taken at the same time according to the technique described previously, and always between 9 and 11 in the morning.

All marrow punctures were made in the dorsal iliac spine, a site which is psychologically preferable to the sternum, particularly under the prevailing conditions (see above concerning traditional views of blood loss). After careful and reassuring information to each volunteer individually, and following local anesthesia, about 0.5—1 ml marrow was quickly aspirated with a bone marrow needle, tightly connected to a 10 ml Luer-Lok syringe. The needle was rotated during the aspiration to ensure that enough marrow was obtained. The marrow was ejected onto a concave glass and the marrow particles were moved up to the edges of the glass. 6—8 slides with a "thick film" (squash preparation) were made from each aspirate.

The conventional method of staining for hemosiderin was used. The film is fixed in formaldehyde-ethanol for 1—3 minutes (1 part 35 % formaldehyde (Merch p.a.) to 9 parts 95 % ethanol), after which it is washed in distilled water and placed for 5 minutes in 10 % potassium ferrocyanide (Merch p.a.). To this is added 1/2 volume 10 % iron-free HCl and rapid mixing is performed by blowing air through a pipette into the solution. The slide is kept there for 30 minutes, after which it is washed in distilled water and dried. All equipment used was carefully cleaned and washed in deionized water. The potassium ferrocyanide was prepared fresh for each staining.

The preparations were classified into six grades according to a modification of the method of Rath

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* I am indebted to Ato Mebrato Yohannes, Director of the Home for Delinquent Boys, and to Colonel Alemayehu, head of the ammunition factory, for their kind cooperation and assistance.

** I am grateful to Dr. K. Weithaler, head of the Body Guard Hospital, and his staff for permission to examine and test his patients and for their assistance.

Table 13. *Number of subjects, mean age and age range in different hemosiderin grades.*

Hemosiderin grade	0	trace	+	++	+++	++++	Total
No. subjects	3	11	25	36	26	35	136
Mean age	4.3	12.7	18.0	19.7	26.2	35.5	
Range	3—6	3.5—20	3.5—40	9—65	7—55	16—60	

and Finch (93): 0, trace, + (slight), ++ (moderate), +++ (moderately heavy) and ++++ (heavy or very heavy).

They were examined and assayed independently by two examiners (M.P.* and Y.H.) who did not have access to each others' results. In 17 cases there was a divergence of one grade and in 2 of two grades (M.P. > Y.H. in 10 cases and Y.H. > M.P. in 9 cases). These were reviewed a second time and decision made regarding grouping. In view of the fact that the method involves subjective evaluation the result must be considered satisfactory.

Results

The number of subjects, the mean age and age range in the different hemosiderin grades are given in Table 13.

As is clearly demonstrated there was an increasing hemosiderin deposit with increasing age, although with considerable overlapping.

Fig. 8 shows the percentage of grades +++ and ++++ in different age groups. Here again it can be seen that with increasing age, the number of subjects with an increased hemosiderin load in the bone marrow became higher. In the age group 20—29 years slightly more than half the subjects (52.8 %) were given a score of +++ or ++++, whilst about four-fifths (81.6 %) of those ≧30 years of age were given this score.

In no case below 10 years of age was a score of ++++ noted. In the age group 10—19 years this score was recorded in 2.8 %, in the group 20—29 years in 36.1 % and in the group ≧30 years in 55.3 %.

Chronic longstanding infections may result in increased hemosiderin deposits in the reticuloendothelial cells in the bone marrow (93). In order to minimize the possibility that the increased hemosiderin deposits found in increasing percentage with increasing age were caused to a considerable degree by this mechanism, all subjects with an ESR of more than 20 mm were excluded. The result is seen in Table 14. As will be seen these percentage figures are very close to those in Fig. 8, where all subjects are included.

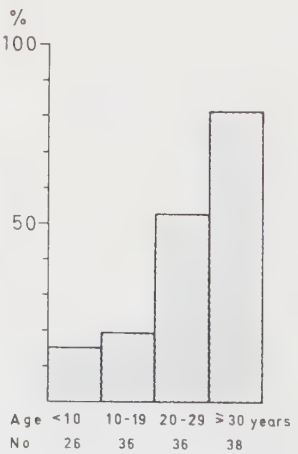

Fig. 8. Percentage of subjects with hemosiderin grades +++ and ++++ (scale 0 — ++++) in different age groups.

* I am very grateful to Mrs. Margit Persson, S.R.N., who has had long experience in bone marrow hemosiderin evaluations, for her kind and generous assistance.

Table 14. *Percentage of subjects with increased hemosiderin load (grades +++ and ++++) and ESR ≤20 mm.*

	<10 years	10—19 years	20—29 years	≥30 years
Number	17	24	35	30
% with grades +++ and ++++	17.6	20.8	54.3	80.0

Correlation between hemosiderin load and certain other hematological parameters.

The means and SD of Hb, PCV, SI, TIBC and % transferrin saturation in the different hemosiderin grades are given in Table 15.

The mean Hb values were lower in the lower hemosiderin grades, reflecting the fact that these grades included subjects aged less than 15 years. The mean PCV values paralleled the Hb values, making the mean MCHC agree well with that presented above for healthy and apparently healthy subjects in Addis Ababa (Chapter III). On the other hand, the mean value for ESR gradually diminished in the higher hemosiderin grades. This reflects the higher prevalence of raised ESR in younger subjects which has also been discussed previously.

20 subjects were anemic according to previously mentioned WHO criteria. 14 of those (70.0 %) were teenage boys from the Home for Delinquent Boys. All were marginally low. ESR was above 20 mm in 10 of those 20 anemic subjects as against 13.0 % in the non-anemic.

The mean serum iron values in hemosiderin grades +++ and ++++ were higher than in the lower hemosiderin grades. The differences between the mean value in grade ++++ and those in grades 0 — trace, + and ++ were highly significant while that between grades ++++ and +++ was not significant.

The mean values for TIBC showed only minor fluctuations in groups + — ++++, while the value for group 0-trace was slightly, although not significantly higher.

Consequently, the transferrin saturation was higher in the groups +++ and ++++, reaching a value of 49.8 % in group ++++.

Table 15. *Hematological values in different hemosiderin grades.*

Hemosiderin grade		Hb	PCV	ESR	SI	TIBC	% satur.
0-trace	Mean	13.8	43.5	17.9	70	309	23.0
	S.D.	± 1.64	± 5.53	±12.86	± 35	± 59	±12.2
	No.	14	14	14	11	11	11
+	Mean	14.7	47.3	14.0	86	283	30.8
	S.D.	± 1.21	± 4.13	±10.38	± 37	± 43	±12.6
	No.	24	24	24	22	22	22
++	Mean	14.8	47.3	13.6	82	295	28.4
	S.D.	± 1.52	± 4.12	± 9.65	± 37	± 35	±12.0
	No.	35	35	34	32	31	31
+++	Mean	15.0	48.3	12.6	110	284	38.4
	S.D.	± 1.45	± 4.47	± 9,84	± 39	± 32	±12.5
	No.	24	22	24	22	22	22
++++	Mean	15.5	50.9	8.8	140	284	49.8
	S.D.	± 0.99	± 3.44	± 6.21	± 62	± 35	±22.7
	No.	32	32	33	33	33	33

Table 16. *Hematological values in subjects ≥20 years with ESR ≤20 mm in different hemosiderin groups.*

Hemosiderin grade		Hb	SI	TIBC	% satur.
Trace — ++	Mean	15.8	110	297	37.1
	S.D.	± 1.26	± 33	± 29	±11.0
	No.	22	20	20	20
+++	Mean	15.7	121	289	41.5
	S.D.	± 1.40	± 34	± 33	± 9.9
	No.	13	13	13	13
++++	Mean	15.6	145	282	52.2
	S.D.	± 0.92	± 61	± 35	±22.1
	No.	29	30	30	30

As it is obvious that the mean values of several parameters are influenced by age and thereby also, as has been discussed earlier, by possible subclinical infections as judged from raised sedimentation rates, those with an ESR higher than 20 mm (24 subjects) and also the subjects below 20 years of age were excluded. In 6 cases the ESR was not recorded. These were also excluded. The result is seen in Table 16.

As can be seen the mean values for SI and transferrin saturation gradually increased with increasing hemosiderin deposits, while the mean TIBC remained at about the same level. The difference in SI between the lowest and highest hemosiderin group was almost significant. The mean Hb was similar for all groups and agreed with the mean for adult healthy and apparently healthy males (see Chapter III). 30 subjects (46.2 %) had a hemosiderin grading of ++++.

Comments.

Absence of stainable hemosiderin in the bone marrow was noted in only 3 cases (2.2 %). These were all young (3—6 years). In all the others it was present and in 44.9 % there was an increased load (+++ and ++++). Such a heavy hemosiderin load was noted even at early ages; the youngest subject with a score of +++ was 7 years old and the youngest with a score of ++++ was 16 years. Generally there was an increasing hemosiderin load with increasing age, +++ and ++++ being noted in 81.6 % of those ≥30 years. All the subjects were medically fit, and not suffering from any longstanding chronic infections, although 18.5 % had an ESR above 20 mm. Even after these had been excluded the same relative percentage of increased load persisted in all ages.

Increased bone marrow iron deposits may occur principally when there is a shift of hemoglobin iron into tissues, as occurs in any anemia not due to blood loss or to insufficient iron intake, when there is impaired release from the reticuloendothelial system, as occurs in infection, or when there is an absolute increase in storage iron (8). It is highly unlikely that any of the first two mentioned mechanisms should be in operation. It is, however, quite conceivable that there was an absolute increase in the body storage iron. When the stores are increased there is usually a corresponding increase in marrow hemosiderin, but discrepancies do occur, since iron at this site is only a reflection of reticuloendothelial deposits and is not indicative of the amounts of parenchymal iron present (8, 49).

No less than 46.2 % of subjects 20 years or more with ESR ≤20 mm had a hemosiderin score of ++++ (Table 16). This is a totally different distribution from that reported for healthy adults in the United States and Sweden (8, 48). Hansen and Weinfeld (48) found in 26 hematologically normal

males with an average age of 59 years, that with a hemosiderin scale of 0 to +++, 4 were scored 0, 19 + and 3 ++. None were graded +++. Weinfeld (121), however, in 15 control males, found a significant correlation between total non-hemin iron in the marrow and age, although this was due mainly to a rise in the lower ages.

The finding of increasing serum iron with increasing hemosiderin deposits is in agreement with the findings in conditions of iron overload (8). It was also found in Bantu subjects that there was a stepwise increase in the mean serum iron with increasing severity of hepatic siderosis (50). These increases in mean serum iron were greater and statistically more significant in the Bantu patients without infections. However, Hathorn *et al.* (50) also found a significant elevation of mean TIBC, once appreciable hepatic siderosis was present. This is contrary to the findings in the present study where the mean TIBC remained at about the same level in all hemosiderin groups (Tables 15 and 16).

B. Evaluation of the iron stores by desferrioxamine

During the past few years desferrioxamine (DF), capable of chelating body iron, has been used extensively (25, 26, 43, 44, 45, 49). Studies made by Hallberg *et al.* (43, 44) indicate that the iron excreted with DF does not originate from iron bound to transferrin and that it does not appear to be related to the red cell catabolism. On the other hand, Hallberg *et al.* (45) found a very high correlation between the increase of urinary iron excretion after administration of DF and the concentration of total non-hemin iron in the liver. Harker *et al.* (49) also found a good correlation between chelate iron excretion and liver parenchymal iron, but not reticuloendothelial iron. It was possible to dissociate these storage sites and to show that patients with only reticuloendothelial iron overload and high serum iron saturation did not excrete a significant amount of chelate iron. The results were considered to indicate that DF enters liver parenchymal cells, where it binds excess storage iron. About two-thirds of the intracellularly formed ferrioxamine passes into the plasma to be excreted via the kidney into the urine and about one-third is excreted by way of the bile into the feces.

In the present study an attempt was made to obtain information, by means of the DF test, regarding the magnitude of the body iron stores in male subjects in whom bone marrow hemosiderin was also studied.

Material and methods.

19 apparently healthy Ethiopian male subjects, aged 20—52 years, had a bone marrow aspirate stained for hemosiderin as described earlier. All 19 were also included in the bone marrow study, presented above. Determinations of Hb, PCV, ESR, SI and TIBC were made according to methods described in Chapter III. Nine of the subjects collected a 24-hour urine specimen about 1 week prior to the DF test. In 7 cases this collection was complete. At 8 a.m. an intramuscular injection of 500 mg Desferral® (CIBA), dissolved in 10 ml distilled water was given after the subjects had emptied the bladder. The urine was then collected during the next 6 hours only, as it was not considered feasible to obtain a complete 24-hour specimen. The urine samples were collected in polyethylene bottles, which had been carefully washed and rinsed with deionized water.

The urine specimens thus collected were analysed for iron according to a method described by Hallberg *et al.* (43).

Results.

The hemoglobin concentrations ranged between 14.7 and 17.5 g % (mean 16.2±0.95 g %) and thus none of the subjects were anemic according to previously discussed criteria (in one case the Hb was not recorded, but the PCV value was 52 %, not suggestive of anemia). The mean PCV and MCHC values were also very close to those reported previously for adult healthy and apparently healthy males in Addis Ababa (Chapter III). All had an ESR of 20 mm or less. All the subjects had stainable hemosiderin in the

bone marrow — two had a "trace", one +, one ++, three +++ and twelve ++++ (see grading on page 38).

In the group with high hemosiderin scores (+++ and ++++) the mean SI was higher (162±60 µg %) and the TIBC lower (284±31 µg %) than in the group with low scores (SI 109±26 µg % and TIBC 304±42 µg %). These differences were not significant, however.

The iron excretion in the 7 subjects from whom a complete 24-hour urine specimen was obtained before the DF test ranged between 0.00 and 0.13 mg.

The mean value for the 6-hour iron excretion after DF in the high hemosiderin group was 0.50±0.307 mg, and was not significantly higher than that in the low hemosiderin group, 0.32±0.045 mg.

In the high hemosiderin group the total 6-hour iron excretion ranged from 0.18—1.43 mg and in the low group from 0.26—0.37 mg. The median values in these groups were 0.42 and 0.32 mg, respectively. The highest excretion value, 1.43 mg/6 hours, was reached by a 44-year old healthy man. He had an SI value of 307 µg %, a transferrin saturation of 90.6 %, and a hemosiderin score of ++++. The lowest excretion, 0.18 mg, was noted in a 50-year old man, also with a score of ++++. His SI value was 168 µg % and transferrin saturation 62.0 %. See Fig. 9.

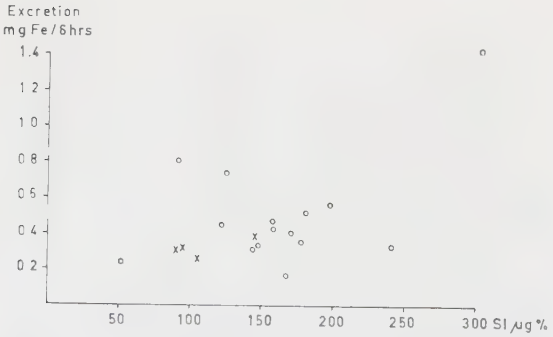

Fig. 9. Iron excretion after 500 mg DF in relation to serum iron in 19 males; 4 with hemosiderin grades trace — ++ (×) and 15 with grades +++ and ++++ (o).

The amount of urine voided was quite low in several cases (hot season). Two subjects with the lowest iron excretions in the high hemosiderin group voided only 168 and 190 ml, respectively. One man misunderstood the instructions and manged to drink 3 liters, voiding 1,640 ml (iron excretion 0.34 mg). The others voided between 145 and 880 ml. The iron excretion per ml voided urine in the low hemosiderin group was 0.92 µg and in the high group 1.47 µg. The difference is not significant.

Comments.

In normal male subjects the mean 24-hour iron excretion after administration of DF in a dose of either 500 mg or 10 mg per kg body weight has been reported to be between 0.59 and 0.72 mg (6, 43, 44, 45, 49). The maximal excretion seems to occur 3—4 hours after the DF injection (43); the mean excretion during the first 6 hours corresponds approximately to half the total 24-hour excretion (6, 43), and has been reported to be between 0.29 and 0.40 mg (6, 43, 96) in normal males. In hemochromatosis, iron excretion values of above 20 mg in 24 hours have been reported (49). Rosen *et al.* (96) reported values ranging between 1.2 and 7.0 mg in 6-hour urine specimens in patients with hemochromatosis. In transfusional hemosiderosis a 24-hour excretion of about 10 mg iron has been found (49).

The iron excretion in the subjects with low — normal hemosiderin scores (trace — ++) in the present series lay entirely within reported normal ranges. However, the excretion in the subjects with high hemosiderin scores (+++ and ++++) was not impressive. In 9 of the 15 males a value of 0.40 mg or more was noted and in only one case did it exceed 1 mg (1.43 mg).

Several subjects had a low diuresis probably due to the rather high environmental temperature and some reduction in iron excretion has been reported with fluid restriction (70),

although this mechanism can hardly account for a considerably reduced excretion.

In evaluation of the results of the DF test the following seems relevant. The reticuloendothelial cell may be regarded as the normal regulator of serum iron and the normal site of iron stores (49). Any influx of excess iron is compensated for by a corresponding increase in reticuloendothelial iron but in some iron overload states, for reasons that are not entirely clear, regulation by the reticuloendothelial cells is inadequate, in which case serum iron rises and parenchymal iron deposition occurs (49).

In idopathic hemochromatosis the iron deposits in the RES cells are not prominent, while in the iron overloaded Bantu they can reach considerable proportions (8), the emphasis with increasing liver iron being on reticuloendothelial involvement (19).

The state of the liver parenchymal iron stores in the subjects under study was not known, although there may be reason to believe that they were probably not excessive (see below). The mean SI in the high and low hemosiderin groups did not differ significantly although the latter group comprised only 4 subjects. There was, however, a highly significant difference in mean SI between the high hemosiderin group in this DF study and the low (trace — ++) group with 20 subjects in the bone marrow study (see Table 16), while the mean TIBC was about the same in both these groups. The raised SI and transferrin saturation in the high hemosiderin group and the slightly although not significantly higher urinary iron excretion after DF might be suggestive of an incipient parenchymal cell overload (49).

Harker *et al.* (49) postulate in their study that iron for both urinary and fecal excretion mobilized by DF is derived from the iron deposits within liver parenchymal cells and conclude that the DF test is useful for detecting significant liver parenchymal iron overload. If this is accepted and applied to the results in the present study the conclusion is that increased hemosiderin deposits found in the bone marrow represent a mainly reticuloendothelial iron overload state in the body.

C. Liver iron in autopsy material

As mentioned previously the iron content of the Ethiopian diet is often extremely high, in adults in the order of 300—500 mg/day. The situation is thus similar in this respect to that in the Bantu population in Southern Africa, although the availability of the iron in the Ethiopian diet may be much lower than that of the Bantus. The absorption of iron from Bantu beer has been found equal to that of a ferric salt (12).

The studies of hemosiderin in the bone marrow indicated an increasing reticuloendothelial load with increasing age — for the age group ≧30 years definitely above the Western standard. It thus seemed of interest to study by chemical methods the iron content of the livers of Ethiopian subjects.

As was mentioned earlier (see "Introduction" to this Chapter), several studies among Bantus have revealed a high incidence of siderosis in autopsy or biopsy material. Bothwell *et al.* (7) found in an analysis of 147 Bantu subjects, aged 11—70 years (131 of them males), who died from accidents, that 89 % had varying degrees of siderosis (liver iron ≧100 mg/100 g dry weight). In 48 % the siderosis was of a mild degree (100—390 mg/100 g) but in 19 % the liver iron concentration exceeded 1,000 mg/100 g. The remaining siderotic cases showed a moderate increase (400—990 mg/100 g) and only 11 % had values below 100 mg/100 g.

In a recent report by Weinfeld *et al.* (122) 27 Swedish control males aged 24—63 years had in biopsied liver material a mean value for non-hemin iron of 80.2 mg/100 g dry weight (range 19.4—227.0). Seven of the subjects or 26 % had iron values above 100

mg. The corresponding value for 16 women was 37.1 mg (range 5.5—106.6).

Material and methods.

Autopsies are made in Addis Ababa mainly on medico-legal indications. Through the courtesy of Dr. E. Codeleoncini at the Department of Pathology at the Menelik Hospital liver specimens were obtained from 107 subjects, representing consecutive autopsies in June and October, 1967. Nine specimens had to be discarded and 98 thus remained for analysis (92 males and 6 females). A total of 76 (70 males and 6 females) had died through road accidents (31), criminal assault (23), suicide (hanging) (9), or suffocation, drowning and similar accidents (13).* No information on previous diseases or nutritional status was available. Two of the persons who died through hanging showed concomitant diseases, 1 (alcoholic?) cirrhosis and 1 exudative pleuritis. They were nevertheless included among the accidental and violent deaths. The main causes of death among the 22 who had died of disease was heart failure (9), infections (6), cerebral hemorrhage (3), internal bleedings (2), pulmonary cancer (1) and uremia (1).

The liver specimens were stored deepfrozen until analysed for nonhemin iron according to a method described by Weinfeld (121). The analyses were performed at Dr A. Weinfeld's laboratory at Gothenburg under his supervision. The results are expressed as mg non-hemin iron/100 g dry liver weight.

Results.

The results for males are summarized in Table 17. The age of the males who died from trauma ranged between 8 and 70 years. The highest mean value for liver iron was found in the age group 20—29 years and the lowest in the 30—39 year group (almost significant difference). The mean value in the groups <20 years and ≧40 years did not differ significantly. The mean value for all subjects ≧20 years and who died from trauma did not differ significantly from that in the subjects who died from disease (age range 20—70 years).

In subjects ≧20 years who died from trauma the nonhemin iron ranged from 8.2—266.0 mg/100 g and 26 out of 65 subjects, or 40.0 %, had values ≧100 mg. In the male subjects who died of disease the range was 13.1—213.3 mg/100 g and 8 subjects or 36.4 % showed values exceeding 100 mg.

All 6 females had died through accidents. Their ages ranged between 3 months and 70 years and the non-hemin liver iron ranged from 22.9—103.9 mg/100 g.

Table 17. *Liver iron (mg Fe/100 g dry liver weight) in males who died from trauma and in males who died of disease in different age groups.*

Age	Mean and SD	<100 mg No	%	100—199 mg No	%	200—299 mg No	%	Total number
				Died from trauma				
<20 years	79.8 ±55.48	4	80.0	1	20.0	0	—	5
20—29 years	105.4 ±72.30	17	54.8	10	32.3	4	12.9	31
30—39 years	62.7 ±40.55	14	70.0	6	30.0	0	—	20
≧40 years	84.4 ±59.28	8	57.1	5	35.7	1	7.1	14
≧20 years	87.8 ±63.26	39	60.0	21	32.3	5	7.7	65
				Died of disease				
≧20 years	85.9 ±54.56	14	63.6	7	31.8	1	4.6	22

* These 76 subjects will be referred to in the following as "subjects who died from trauma".

Comments.

The mean values and ranges for non-hemin iron in male subjects who died from trauma are close to those reported by Weinfeld (121, 122) for control Swedish subjects. A total of 40 % of the males who died from trauma had non-hemin iron values ≧100 mg/100 g, which is higher than in the series of Weinfeld *et al.* (122), 26 %, but definitely lower than in the series of Bothwell and Bradlow (7) of Bantu males in which 89 % had values of 100 mg or more. In a study of Mayet and Bothwell from South Africa (75) on 738 livers obtained at autopsy, 554 of which were from white, African and Indian subjects who had died from trauma, the median values in all males were 87, 281 and 60 mg/100 g, respectively (converted to dry weight, assuming a water content of 70 % (121)). The corresponding median values for females were 50, 89 and 39 mg/100 g, respectively. In 708 autopsied adults from Ireland, Israel, Japan, South Africa (white subjects), Boston and San Francisco, Mac Donald and Pechet (73) found that 16 % had liver iron values of more than 100 mg/100 g dry weight.

No information on previous diseases was available for the subjects under study and nothing was known about their food habits and iron intake. It is probable, however, that a significant number came from the poorer strata of the society, with poor eating habits, although no autopsy records were made of gross malnutrition, intestinal helminths or signs of anemia. No fewer than 32 of the males, or 46 % had committed suicide or were victims of criminal assault. There is therefore reason to believe that this series is not comparable as regards health standard and possibly iron intake with the adult healthy and apparently healthy males from whom bone marrow aspirates were taken.

Although it would have been expected to find a higher frequency of high values, the number of low values, on the other hand, was small. Weinfeld (121) found a mean value of 5.5 mg/100 g (range 2.7—10.0) in 6 male subjects with iron deficiency anemia. In the present material only one male, 40 years old and killed in a road accident, came below 10 mg (8.2). Of the females, 2 or possibly 3 were at the menstruating age and showed values ranging from 22.9—53.2 mg/100 g which were well within the range found in menstruating Swedish women (121, 122).

Although the present series is not satisfactory as regards background data of the subjects, and is probably not comparable to that in the bone marrow hemosiderin study, it could be concluded that the non-hemin iron values in the liver specimens were comparable to those in a normal Swedish control material, supporting the view of an adequate but not excessive uptake and retention of dietary iron. Obviously then, the extremely high amounts of iron in the Ethiopian diet are available for absorption only to a limited extent (cf. Chapter II).

Summary

From 136 healthy and apparently healthy subjects aged 3—65 years, bone marrow smears were examined for stainable hemosiderin, which was graded from 0 to ++++. The results indicated that with increasing age the percentage of subjects with increased (+++ and ++++) hemosiderin load rose successively. In ages ≧30 years about 80 % had +++ and ++++ hemosiderin. Only 3 young children had no detectable hemosiderin in the marrow.

In subjects ≧20 years with ESR ≦20 mm there was a successive rise in SI with increasing hemosiderin grades, with an almost significant difference between the lowest and highest hemosiderin groups, parallelled by a rise in transferrin saturation.

In 19 adult male Ethiopians, a desferrioxamine (DF) test was made in which 500 mg DF was given intramuscularly. Four of these subjects had hemosiderin grades trace — ++, and 15 had grades +++ to ++++. The mean values for 6-hour urine iron excretion were 0.32 and 0.50 mg, respectively, a non-significant difference. There was considerable overlapping between the excretion in the two groups, and the two extreme values, 0.18 and 1.43 mg, were both reached by older men with a hemosiderin score of ++++ and high serum iron and transferrin saturation values.

For reasons discussed it is suggested that the increased iron deposits found in the bone marrow may have represented a mainly reticuloendothelial iron overload state in the body.

98 autopsy liver specimens were analysed for non-hemin iron. 76 were from persons who died from accidents or violence (70 males and 6 females) and 22 from subjects who died from disease (all males). The mean value for non-hemin iron was very similar in both these groups (for males ≧20 years 88 and 86 mg/100 g dry liver weight, respectively) and corresponded to that found in normal Swedish males.

Among all males ≧20 years, 39 % had values ≧100 mg/100 g, the highest value being 266 mg, while only 1 was below 10 mg.

There was a high incidence of suicide and homicide in the series and there seems reason to believe that it cannot be compared to the healthy and apparently healthy males in whom marrow hemosiderin was studied.

Evidence presented supports the view of an adequate or more than adequate but not excessive uptake and retention of dietary iron and that the extremely high amounts of iron in the Ethiopian diet thus seemingly are available for absorption only to a limited extent.

CHAPTER V.

Hematological investigations in a representative Ethiopian highland village

Tropical anemias*

There is abundant evidence that anemia is present among large sectors of the population in tropical countries. In many areas and especially among children and women of fertile age it may reach appalling degrees and frequencies (104, 107). Etiologically, dietary deficiencies, infections and parasitic infestations are by far the most common causative factors (62), although in certain areas anemias of other causes such as sickle cell anemia or other hemoglobinopathies are prevalent (123).

Although deficiencies of several essential nutrients result in anemia, those deserving primary consideration are deficiencies in iron, vitamin B 12, and folic acid (89). The same type of anemia may result from dietary deficiency *per se*, poor absorption and utilization and/or increased requirements such as occur in rapid growth and in pregnancy. Associated parasitism may enhance the significance of one or more of these factors (89).

The morphological picture of a fully developed iron deficiency anemia is characterized by hypochromic and microcytic erythrocytes, low levels of serum iron, raised transferrin levels with a low transferrin saturation and absence of stainable hemosiderin in the bone marrow. The typical blood picture in anemia caused by deficiency of vitamin B 12 or folic acid is one of macrocytosis with a megaloblastic bone marrow. The term "dimorphic" has been proposed to refer to cases in which both macrocytic and hypochromic microcytic anemia exist at the same time as a result of mixed deficiency (Trowell, quoted by (123)).

Studies carried out in India, Africa and Central and South America have shown that anemia due to iron deficiency is the most common in these regions (62). Although in most tropical countries the dietary iron intake may be equal to or greater than that accepted for adequate diets in North America and Europe (62, 88), several factors may interfere with the absorption. The diets are generally rich in bulky carbohydrate foods from which iron absorption is low (58, 80) and may have high contents of phosphates and phytates with low calcium levels (23), which may interfere with the iron absorption, although the views are conflicting as to whether or to what extent this interference plays a role (8).

Important factors in the development of iron deficiency anemia in females are repeated pregnancies and lactation, when the demands for nutrients exceed those required to maintain health in a non-pregnant woman, and where high frequencies of such anemia have been reported (89, 107).

Some controversy exists as regards increased losses of iron through the skin by sweating in hot climates (8) .There seem to be indications that under adverse temperature conditions, the basal loss of about 0.2 mg per day may be increased to 0.5 mg (9).

The association of hookworm infection and anemia has long been recognized, although apparently there has to be a certain worm load, as measured by the number of eggs per gram feces, before a significant decrease of the hemoglobin concentration is achieved. This critical level was found to be 5,000 eggs

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* This review does not pretend to cover the enormous literature dealing with this subject, but to serve mainly as an introduction to this chapter.

for adult men and 2,000 for children and women (68), reflecting the greater vulnerability in the latter groups where growth and an increased physiologic iron drain raise the demand for iron. 5,000 eggs per gram feces correspond to about 250 worms, meaning a loss per day of about 10 ml blood, containing about 5 mg iron out of which 3 mg would be lost in the feces after re-absorption of the rest (68). There are great individual variations, however. An important factor determining the development of anemia is the dietary iron content (29), and it has been demonstrated that hookworm anemia can be controlled by iron without deworming (35).

Hookworms are among the most important causes of ill health in many developing countries and constitute a formidable public health problem (29).

Nutritional megaloblastic anemias in the tropics are comparatively much less frequent than iron deficiency anemias. Both sexes may be affected but women predominate and these anemias are far more common in pregnant than in non-pregnant women (89). Most of them respond to treatment with folic acid, and some to vitamin B 12 (88). Studies in India in pregnant women showed that although the majority of the anemia cases were due to iron deficiency there were indications that 25—40 % may have had concomitant deficiency of folic acid and/or vitamin B 12 (89).

Vegetable foodstuffs do not contain vitamin B 12, and in vegetarians this vitamin is presumably obtained through activity of intestinal micro-organisms (88). Folic acid is present in appreciable quantities in green leafy vegetables, legumes and cereals; any cereal diet would give an adequate daily intake (88), and it remains obscure which other factors may be operative in producing folic acid deficiency anemias. Cooking losses, increased demands during pregnancy and malabsorption have been suggested causes (88).

Protein—calorie malnutrition is exceedingly common among infants and preschool children in developing countries, and the most severe form, kwashiorkor, is usually combined with a mild to moderate anemia, also in the absence of other obvious anemia causes (65). Anemia has also been described in cases of cystic fibrosis with protein failure, without other causes of anemia, and responding to high protein feeding before the addition of pancreatic enzymes (102). Animal experiments (46, 105) with low protein diets resulted, in rats (46), in a slight fall in the hemoglobin concentration but a large fall in total hemoglobin, the anemia being microcytic and normochromic or slightly hypochromic, while in the Rhesus monkey (105) a moderate anemia of the normocytic and normochromic type developed. Doubts regarding the part played by protein malnutrition in the production of anemia have arisen as a result of the poor hematological response which commonly follows the administration of protein to patients with kwashiorkor or marasmus. The anemia is ascribed to diminished erythroid activity in the bone marrow (3, 103) which has been interpreted as an adaptation to undernutrition (103). The role of moderate protein malnutrition in the production of anemia is, however, not clear (88).

A mild non-progressive anemia frequently accompanies various chronic infections and systemic diseases, usually normocytic and normochromic but sometimes hypochromic or even microcytic (17, 123). The hypochromia is usually not as marked as in iron deficiency anemia and the reticuloendothelial iron in the bone marrow is normal or increased. The serum iron is low and the transferrin levels decreased. The anemia is caused by a modest shortening of the erythrocyte survival time for which the bone marrow fails to compensate by increased production (17). Except for severe acute infections, especially in small children, infections of less than a month's duration are not, as a rule, accompanied by significant anemia and it has been

suggested that the previous nutritional status of the patient may possibly be of some importance in determining whether or not anemia develops (18). The interaction of nutrition and infection has been well documented (99). Although a variety of viral and bacterial infections are exceedingly common in tropical countries the exact role played by the infection in producing anemia has not been satisfactorily demonstrated. Increased sedimentation rates are often noted in tropical Africa (113) and are usually attributed to malnutrition and infection. When, in apparently healthy Bantu males, those with an ESR above 8 mm were excluded, the hemoglobin levels of the remainder were similar to those of Europeans with the same low ESR (113). In this connection it might be appropriate to point out that optimum hematological values in tropical and temperate zones are the same (62) and that no racial differences occur, although other opinions have been expressed (20).

Malarial infection of a slight degree may not cause anemia. Longstanding untreated infections cause a hemolytic anemia which is more severe in malignant tertian malaria. In uncomplicated cases the response to treatment is extremely rapid. In hyperendemic regions, children have a rising parasitic count from birth to the second year, coinciding with the time when the immunity is lowest, after which there is a fall (113).

The hemoglobinopathies of public health importance are those characterized by hemoglobins S, C and E, and the thalassemias. The gene for Hb-S is widely distributed in equatorial Africa but limited to the north by Sahara and the Ethiopian highlands (52). Hb-C is found in West Africa, while Hb-E is seen primarily in the tropical regions of South-East Asia, and the thalassemias have their greatest known prevalence in the Mediterranean regions and in South-East Asia (52).

The hemoglobinopathies caused by Hb-S, Hb-C and Hb-E in the homozygous form give rise to hemolytic anemias, the first mentioned, sickle cell anemia, presenting the most severe forms. In the heterozygous state, the sickle cell trait may present symptoms under certain circumstances while the other two have no known clinical effects (52).

The village Ijaji

In the beginning of 1963, longitudinal studies of children 0—10 years old started in the village Ijaji, 220 km west of Addis Ababa (77). The village was chosen after careful surveying and is considered typical of and representative for the living conditions in the Ethiopian highlands. It had at that time about 1,600 inhabitants, but has since grown considerably. In October 1966 when the last census was made it had 2,100 inhabitants. The size was therefore considered suitable for nutritional studies.

Fig. 10. The main road through Ijaji.

Approximately 25 % of the heads of households are farmers and 25 % traders, a reflection of the fact that the village is located along a main road in an agricultural region. About 20 % are bar-owners or subsist by producing beer or local liquors; most of these are women.

80 % are Orthodox Christians and the remainder Moslems. The predominant ethnic group is the Galla tribe (60 %), followed by the Amhara (30 %). A little more than 10 % of the families are polygamous. In

1966, when the most comprehensive census was made, 43 % of the population were below 15 years. 54 % were females, with the highest predominance in the ages 20—40 years (56).

In the farming areas of the village, the typical house is a circular hut with a thatched roof. Several such huts are gathered in fenced compounds. The huts have an earthen floor and no windows, and usually have a fireplace on the floor in the middle of the hut. Chicken, calves and other animals are not seldom kept in the living houses.

The houses along the road and around the market place are usually of better standard, they are rectangular and most often have a roof of corrugated iron.

Water is available all the year round from two covered wells and from three streams.

In 1962, a health station was started, mainly concerned with curative work. The nearest hospital is 90 km away.

An elementary school started in 1962. From a modest start the number of pupils has grown and in 1967 was about 250 in grades 1—6. It can therefore be expected that the degree of literacy will increase. In the census of 1966, 75 % of the heads of households and 98 % of the wives were illiterate.

Material and methods

In November 1965, 433 children of ages 0—11 (—12) years were clinically examined in Ijaji to obtain a base-line material before the start of an applied nutrition program. These corresponded to approximately 75 % of all children in this age group in the village. 402 children or 92.8 % were tested hematologically, which must be considered very satisfactory under the prevailing conditions. A stool sample was obtained from 346 children. 304 of these were tested hematologically.

In December 1965, 100 school children, aged 4—20 years, were examined in a small village, Backo, 45 km west of Ijaji. This village greatly resembles Ijaji and is located at the same altitude. The school had started a few months earlier in very primitive premises and comprised grades 1 and 2. The children can be considered representative of the village, and all children in the school were examined, all had a blood sample taken and most of them also had urine and stool analysis. 52 of these 100 children, aged 10—14 years, were included in the present study to obtain full age continuity. 45 children tested hematologically in Ijaji were 10—12 years. These are included in the 10—14 year group unless stated otherwise.

Thus the study concerns a total of 454 children aged 0—14 years. Up to 10 years there was about the same number in each sex (177 boys and 180 girls). In the 10—14 year group, boys predominated (69 boys and 28 girls).

In November 1967, 416 persons aged 15 years and older were called for examination in Ijaji according to a predecided geographical sampling system to ensure that all areas and thereby the main professions would be equally represented (farmers and traders). 271 or 65.1 % attended and had an abbreviated clinical examination. Of these, 196 or 72.3 % were tested hematologically. 15 persons refused blood test and for the remainder there was no laboratory capacity.

During the past few years when longitudinal studies were in process, the farmers had been, on the whole, co-operative while certain trading areas had shown some resistance. The fact that about 75 % of the children who were called for examination attended, means, however, that a very good representativity was achieved. The precise figure cannot be given as the census was made 2 months earlier and the mobility is very high. As for the adults, the distribution of the clinically examined persons regarding sex and religion corresponded closely to that found in the last census made during the fall of 1966 — 44 % of the subjects above 15 years were males and 81 % (of heads of households) were Orthodox Christians, the remainder Moslems. In the clinically examined material there was, however, an over-representation of farmers (46 %) compared to the census (28 % of heads of households in the census), indicating a poorer attendance of people belonging to other occupational categories.

The attendance figure of 65.1 % of the adults has to be considered satisfactory, especially in view of the fact that it was announced that blood testing was to be done, although on a voluntary basis.

Capillary blood for determination of hemoglobin, packed cell volume and sedimentation rate was taken from all children referred to above, after previous warming and cleaning of the hands in warm water. The determinations were made according to methods described previously.

The subjects aged 15 years and older had venous blood taken by means of a Vacutainer, according to a previously described technique. Unfortunately 60 blood samples for Hb, PCV and ESR from adults had to be discarded because of a technical mishap.

Blood for determination of serum iron and transferrin was taken from 117 adults at the same time (although in different tubes), according to a previously described technique and always between 9 and 11 a.m. These 117 persons represented all cases from whom blood samples were taken between 9 and 11 a.m. After clotting, the samples were spun and the serum separated and kept in a field deep freezer for 1—2 days before being taken to Addis Ababa for processing.

From 17 females and 1 male (= all attending and agreeing to blood tests on one particular afternoon) blood was taken for determination (on serum) of folic acid and vitamin B 12. This serum was kept deep-frozen for about 1 month, after which it was processed at the Institute of Medical Chemistry in Uppsala, Sweden.

81 stool samples were obtained fram the 271 adults who had a clinical examination.

In May 1967, 30 blood samples for serum iron and transferrin determinations (and at the same time for Hb, PCV and ESR) were obtained from children ≥ 5 years in Ijaji at a follow up examination there and 46 blood samples for the same purpose from Backo school children aged 5—14 years. All these samples were taken by means of Vacutainers (also for Hb, PCV and ESR) using the same technique as described earlier and with the same time precautions (9—11 a.m.). The 30 samples from Ijaji represent all children attending the last morning of examination and the 46 from Backo all children examined at that time in the school in this age group.

On all blood-tested subjects, a thick blood film was examined for malaria. (This was negative in all cases).

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For the sake of simplification the subjects from Ijaji and Backo are referred to in the following as from Ijaji only.

Results

The mean values and SD of the determinations of Hb, PCV, MCHC and ESR are presented in Table 18, and the relative distributions of Hb, PCV and MCHC in Figs. 11, 12 and 13 (infants excluded). For comparison the corresponding mean values from the "normal material" in Addis Ababa are given.

The lower altitude in Ijaji (1,850 m) would justify a deduction of about 0.2—0.3 g % from the mean Hb values in Addis Ababa to make them comparable to the mean values in Ijaji (see previous discussion on this subject). When this deduction was made there was still a marked difference in mean Hb values between the "normal material" in Addis Ababa and those from Ijaji in all age groups. The mean PCV values were slightly less depressed, resulting in a lower mean MCHC in the village population compared to Addis Ababa. The lowest mean MCHC value was recorded in the age group 6—11 months. It should also be noted that in all age groups there was a considerable difference in mean ESR compared to the Addis Ababa material.

In adult males, the highest mean Hb value was recorded in the ages 25—44 years (14.2 g %, n = 35) and the lowest in the group ≧45 years (12.9 g %, n = 13). In the group 15—24 years the mean Hb was intermediate (13.2 g %, n = 8).

In adult non-pregnant females there was a successive decrease with increasing age: 15—24 years (n = 11) 13.2 g %, 25—34 years (n = 22) 12.6 g %, 35—44 years (n = 17) 12.6 g % and ≧45 years (n = 16) 12.1 g %.

Table 18. *Hematological values in different age groups in Ijaji. For comparison mean values from the healthy and apparently healthy subjects in Addis Ababa (cf. Tables 4 and 8).*

Age group		Ijaji Hb	PCV	MCHC	ESR	Addis Ababa Hb	PCV	MCHC	ESR
1—5/12	Mean	11.3	37.8	29.6	28.2	—	—	—	—
	S.D.	± 1.04	± 3.40	± 1.86	± 10.86				
	No.	11	10	10	11				
6—11/12	Mean	10.1	36.7	27.4	25.9	—	—	—	—
	S.D.	± 1.54	± 3.25	± 2.31	± 14.13				
	No.	20	22	20	22				
1—4	Mean	11.1	37.9	29.2	30.3				
	S.D.	± 1.35	± 3.16	± 2.15	± 11.87	12.9	42.0	30.8	17.5
	No.	154	154	149	158				
5—9	Mean	11.5	38.2	29.8	30.1				
	S.D.	± 1.53	± 3.80	± 2.56	± 11.14	13.5	43.8	30.8	13.3
	No.	156	156	151	159				
10—14	Mean	12.4	40.2	30.9	24.9				
	S.D.	± 1.18	± 3.02	± 2.63	± 12.79	13.9	44.8	31.1	12.7
	No.	94	96	94	95				
Ad. males	Mean	13.8	46.0	29.9	15.7				
	S.D.	± 1.63	± 3.93	± 2.27	± 10.47	15.7	51.1	30.9	7.5
	No.	56	56	56	56				
Ad. females	Mean	12.6	42.2	29.8	23.0				
(non-pregnant)	S.D.	± 1.48	± 3.91	± 2.00	± 11.80	14.2	46.0	30.9	16.0
	No.	67	67	67	67				

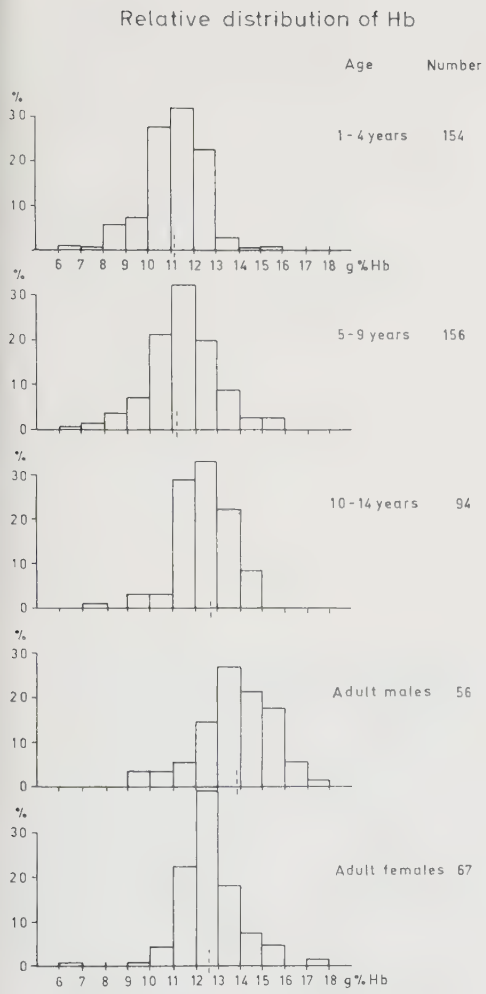

Fig. 11. Relative distribution of Hb in children and adults in Ijaji.

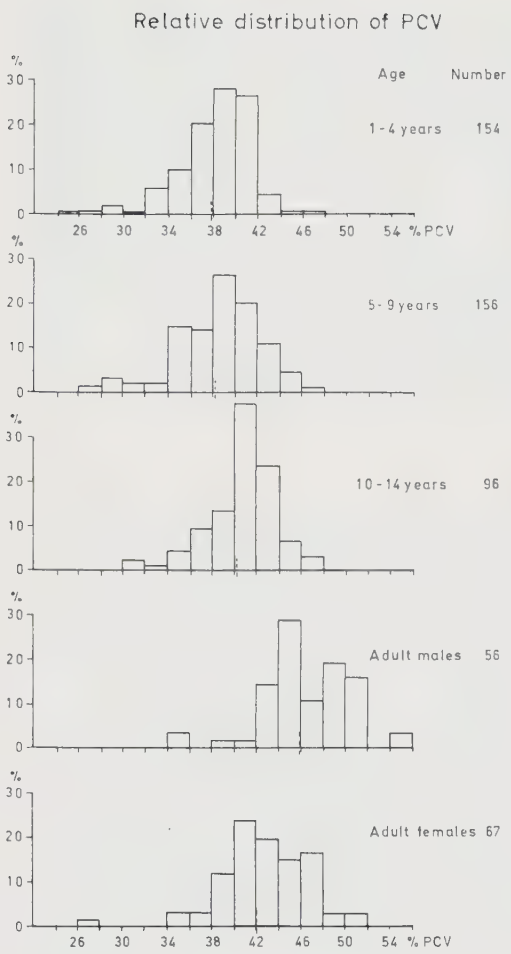

Fig. 12. Relative distribution of PCV in children and adults in Ijaji.

Anemia evalution.

As was discussed previously, evaluation of anemia rates is a difficult task in the absence of fixed limits below which anemia can be said to exist. As this study was part of a public health applied nutrition program in a developing country, based in important aspects on the views of WHO, it was decided to use the criteria of WHO with regard to hemoglobin levels below which anemia "could be said to exist" (see Chapter III). These WHO values were adjusted by +5 % for altitude (actual WHO value × 1.05). Because of the special hematological conditions during the first few months of life, the children below 6 months will not be considered here.

In Table 19 the percentages of anemia according to these criteria are presented. It is obvious that even without any altitude adjustment the anemia rate was very high in all age groups and highest during the second six months of life.

When altitude adjusted anemia limits given

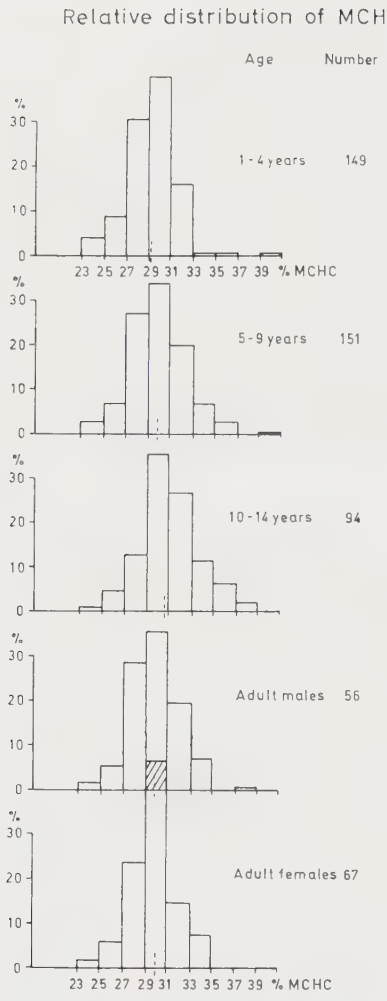

Fig. 13. Relative distribution of MCHC in children and adults in Ijaji.

Table 19. *Percentage of anemia in different age groups in Ijaji according to criteria of WHO. See text.*

	WHO anemia limits not adjusted for altitude	adjusted by + 5 %
6/12—11/12	65.0	80.0
1— 4 years	32.5	48.1
5— 9 years	49.4	68.6
10—14 years	50.0	69.2
Ad. males	26.8	44.6
Ad. females*	29.9	53.7
Total	40.6	59.0

* non-pregnant

by Natvig *et al.* (82, 83) were applied (see Chapter III), the following percentages could be classified as anemic: 5—9 years 55.8 %, 10—14 years 35.1 %, adult males 67.9 % and adult females 70.2 %.

Boys had a slightly higher rate of anemia than girls in the ages 1—14 years. The difference was greatest in the group 5—9 years (77.2 for boys and 59.7 % for girls). For 1—4 years these figures were 49.3 and 46.8 % and for 10—14 years 70.6 and 65.4 %, respectively. The sex distribution in the 6—11 month age group was very uneven (7 boys, 13 girls) and in this group girls were more often anemic than boys (84.6 *vs* 71.4 %).

In the following, 3 main reasons for this high prevalence of anemia will be discussed: anemia due to infections, anemia due to hookworm and nutritional anemia.

Anemia and infection.

During the clinical examination of the children in Ijaji in November 1965 it became evident that there was an exceedingly high frequency of infections of different kinds. In Table 20 some of the common types in children 0—14 years are recorded. The figures refer to the blood-tested children.

A history of gastroenteritis and upper respiratory infection refers to some time during the 2 weeks preceding the examination and is defined for gastroenteritis as 4 or more watery and/or mucous and/or bloody stools per day with or without vomiting; and for upper respiratory tract infection as 3—4 of the following symptoms: fever, difficulties in breathing, sneezing and coughing.

Skin infection refers to bacterial infections such as tropical ulcer, pyodermia, impetigo and the like.

"Chiggers" is an intracutaneously or subcutaneously located infestation with the sand flea, usually under the toes or toe nails, causing a local chronic infection, most often with lymph gland enlargement in the groin.

As can be seen in Table 20, the prevalence of gastroenteritis and upper respiratory infections was high already during the infant year and continued to be high for enteritis

during the pre-school age, after which a decrease was noted. The scabies rate increased with increasing age during childhood, as also did "chiggers", while the skin infection rate was about 30 % after 1 year. These impressive figures illustrate well the poor hygienic standard prevailing in such a rural village, in spite of the fact that water is available all the year round.

The adults underwent an abbreviated form of clinical examination, and the results are not fully comparable with those of the children. However, in all clinically examined adults the rate of scabies was only 5.1 % and skin infection 1.8 %, while axillary and/or inguinal lymph gland enlargement was diagnosed in 21.8 %. Some skin affections may

Fig. 15. Scabies with secondary infection on the hands.

Fig. 14. Scabies with secondary infection on the feet and lower legs.

Fig. 16. Impetigo.

Table 20. *Symptoms and signs of infections in blood-tested children in Ijaji and Backo in November and December 1965. See text for explanation.*

Age	Enteritis	Upper resp. inf.	Scabies	Skin inf.	Chiggers	Other inf.*	Lgl. enlarged	"No infection"
<1 year (33)	27.3	24.2	27.3	12.1	0	15.1	3.0	42.4
1—4 years (161)	36.6	14.3	43.5	28.9	36.6	36.7	34.2	12.4
5—9 years (163)	10.4	4.9	44.8	30.7	63.8	52.1	52.8	4.3
10—14 years (97)	3.1	7.2	52.6	27.8	56.7	26.8	51.5	16.5

* Fungus inf. scalp
Otitis
Signs of lung inf.
Relapsing fever
"Fever of unknown origin" (in a few cases more than one of these infections appeared in the same child)

have been missed as the subjects could not be examined naked. "Chiggers" was not recorded although it was not infrequently seen as was also the case with a variety of scratches and fissures on the feet.

The high mean values for ESR (Table 18) should be regarded in the light of these clinical findings. Already during the first half year of life, mean ESR was nearly 30 mm and throughout childhood it remained high. The lowest mean ESR was recorded in adult men 25—34 years of age (11.9 mm), while in the non-pregnant females there was a tendency to increasing values with increasing age (17.6 mm in the 15—24 year group compared with 29.2 mm in females ≧45 years).

In Table 21 the percentages of subjects with ESR ≦10 mm and with ESR ≦20 mm are given. It will be seen that the great majority in all age groups exceeded 10 mm. In ages 1—9 years only a small fraction had ESR ≦10 mm. The majority, except in adult males, also exceeded 20 mm.

In Fig. 17 the subjects are divided according to the level of their ESR and the mean Hb is calculated for each ESR group. In all age groups there was a decreasing mean hemoglobin value with increasing sedimentation rate. The differences between the highest and lowest ESR groups amounted to 1.4—2.9 g % after 1 year and are highly significant. It should be noted, however, that from 10 years and upwards there were but few subjects in the highest ESR groups. The difference in mean Hb between the lowest and intermediate ESR groups was highly significant for adult males and almost significant for children 5—9 years and adult females, while in the remainder the difference was not significant.

For comparison, the mean Hb in healthy subjects in Addis Ababa is also given. As will be seen there was more than 1 g % difference in mean Hb between those with ESR ≦20 mm

Table 21. *Percentage of subjects with low (≦10 mm) and "acceptable" (≦20 mm) ESR in Ijaji.*

Age group	ESR ≦10 mm	ESR ≦20 mm
<1 year	12.1	39.4
1— 4 years	1.9	18.4
5— 9 years	3.1	20.1
10—14 years	13.7	37.9
Ad. males	35.7	75.0
Ad. females	13.4	47.8
Total	9.5	32.4

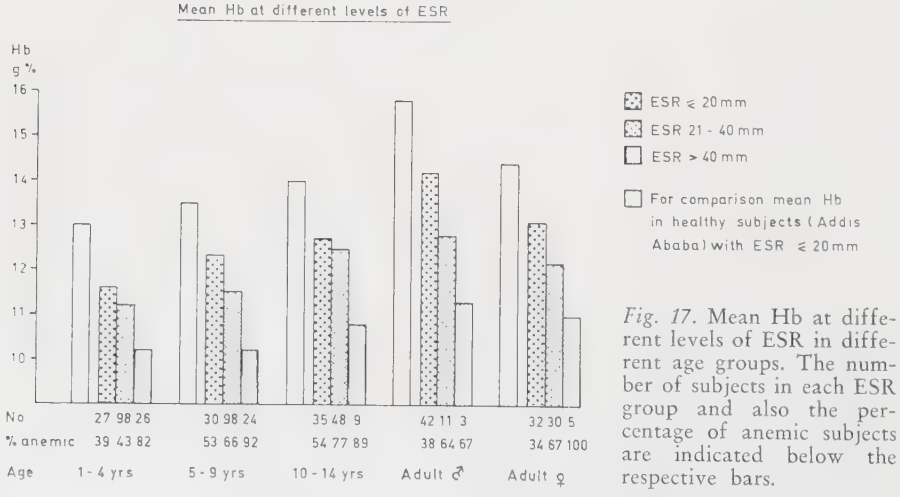
Mean Hb at different levels of ESR
Hb g %
16
15
14
13
12
11
10
No 27 98 26 30 98 24 35 48 9 42 11 3 32 30 5
% anemic 39 43 82 53 66 92 54 77 89 38 64 67 34 67 100
Age 1-4 yrs 5-9 yrs 10-14 yrs Adult ♂ Adult ♀
ESR ≤ 20 mm
ESR 21 - 40 mm
ESR > 40 mm
For comparison mean Hb in healthy subjects (Addis Ababa) with ESR ≤ 20 mm

Fig. 17. Mean Hb at different levels of ESR in different age groups. The number of subjects in each ESR group and also the percentage of anemic subjects are indicated below the respective bars.

in Addis Ababa and Ijaji in all age groups, although the altitude could only account for 0.2—0.3 g %.

In Fig. 17 it is also seen that the percentage of anemic subjects in all age groups was higher in the higher ESR groups and *vice versa*. It should, however, be noted that although this trend was obvious, a considerable proportion of subjects with ESR ≤20 mm were anemic.

Anemia and hookworm.

The mean Hb values in different age groups in subjects with positive and negative microscopic hookworm diagnoses can be seen in Fig. 18. In all age groups the mean Hb value in subjects with a positive diagnosis was lower than in subjects with a negative stool test. The difference, about 1 g % in ages 1—14 years but smaller in the adults, is highly significant in the 5—9 year group, significant in the 1—4 year group, almost significant in the 10—14 year group and not significant in the adults. It is also evident from Fig. 18 that as a consequence the percentage of anemic subjects was higher in the hookworm groups. It should be noted, however, that a considerable percentage in the non-hookworm groups were also anemic. Two infants were diagnosed as having hookworm (20 samples examined). They were 8 and 11 months old with Hb values of 10.3 and 8.2 g %, respectively.

The material in some age groups is rather small. It should also be emphasized that the direct microscopy method does not reveal the extent of the infestation as does the egg counting method.

Nutritional anemia.
a. Iron deficiency.

The anemic subjects were divided into those with MCHC below 27 % and those ≥27 %. This limit was chosen somewhat arbitrarily. It corresponds approximately to the mean minus 2 SD for all age groups in the material of healthy and apparently healthy subjects in Addis Ababa (Chapter III). In the non-anemic subjects in Ijaji none hade an MCHC value below 27 %. In the anemic subjects, on the other hand, a value below 27 % was recorded in a high percentage in the smaller children (see Fig. 19, in which also the *total* anemia rates in the different age groups are recorded). The pre-school age group was divided into children 12—35 months old and those 36—59 months. The rate was highest, 43.8 %, during the second half year of life and decreased gradually during the pre-school years. From 3 years and upwards it ranged between 7.7 and 16.0 %.

In other words, the anemia was found to be of a clearly hypochromic type in a much higher percentage from 6 months to 3 years than after 3 years.

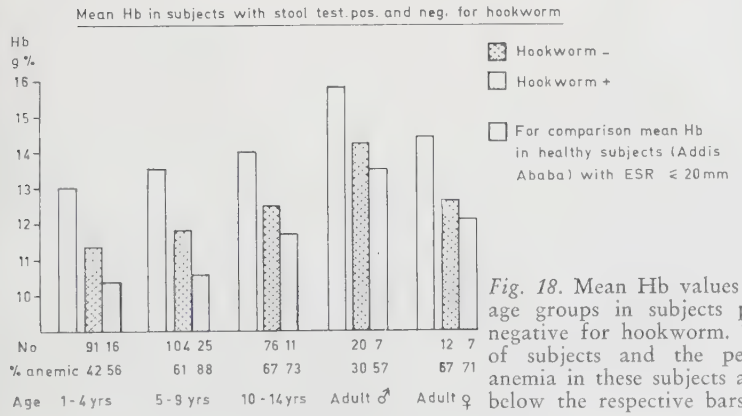
Mean Hb in subjects with stool test pos. and neg. for hookworm
Hb g %
16
15
14
13
12
11
10
Hookworm –
Hookworm +
For comparison mean Hb in healthy subjects (Addis Ababa) with ESR ≤ 20 mm
No 91 16 104 25 76 11 20 7 12 7
% anemic 42 56 61 88 67 73 30 57 57 71
Age 1-4 yrs 5-9 yrs 10-14 yrs Adult ♂ Adult ♀

Fig. 18. Mean Hb values in different age groups in subjects positive and negative for hookworm. The number of subjects and the percentage of anemia in these subjects are indicated below the respective bars.

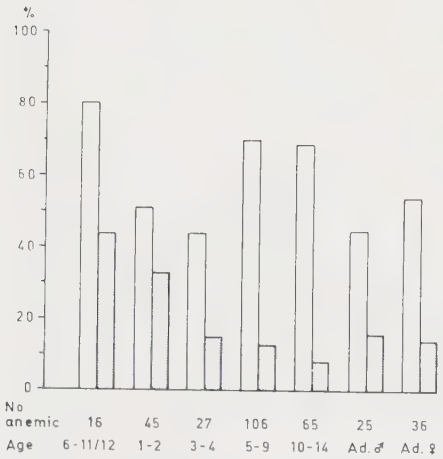

Fig. 19. Percentage of anemic subjects in different age groups (open bars) and percentage of anemic subjects with MCHC below 27 % (shaded bars).

In subjects with a positive hookworm diagnosis 37.5 %, 13.6 and 12.5 % of the anemic cases in the age groups 1—4, 5—9 and 10—14 years, respectively, had MCHC values below 27 %. No adults with hookworm and anemia had MCHC below 27 %. However, rather few adults gave a stool sample. In no case did non-anemic subjects with hookworm have an MCHC value below 27 %.

Therapy trial. During the course of the applied nutrition program, children with frank anemia were given oral iron medication repeatedly. Experiences have shown, however, that in practically all cases the treatment has been interrupted after only a few days or a week due to lack of interest or understanding.

In November 1967, a therapy trial with injectable iron (Jectofer®) was started and all children and adults with frank anemia were called for injections (intramuscular), which were planned to continue for about 1 week. The total dose aimed at raising the Hb to 10—12 g %. 6 weeks later the blood was to be checked again. Unfortunately it was possible to persuade only 5 out of 40 (4 adults and 1 child) to attend and have their blood re-checked, giving an indication of the problems involved in running seemingly unsophisticated programs.

As can be seen in Table 22, four of the 5 subjects improved. Three had a very low initial MCHC, 22—23 %, indicating iron deficiency anemia. One pregnant woman, although responding by improvement, was still considerably anemic at the end of the trial (Hb 8.4 g %), probably due to a too small dose of iron.

Serum iron and transferrin were determined in 3 cases. A 9-year old child displayed the typical picture of iron deficiency (SI 30 µg % and TIBC 396 µg %, saturation 7.6 %), improving after treatment (SI 77 µg %, TIBC 364 µg % and saturation 21.2 %). The second case (no. 140 in Table 22), not responding with raised Hb to iron treatment, had SI and TIBC within mean minus 1 SD for apparently healthy females in Addis Ababa on both occasions (SI 80—83 µg %, TIBC 269—283 µg %).

Table 22. *Result of therapy trial with Jectofer® intramuscularly in 5 subjects in Ijaji.*

No	Sex	Age	Before		After		
			Hb g%	MCHC	Hb g%	MCHC	Hookworm
257.1	M	9	6.6	22.0	10.3	27.8	pos.
204	F*	26	6.8	22.7	8.4	26.3	—
115	F	41	9.9	28.3	12.1	31.0	pos.
156	M	55	10.8	23.0	12.4	29.5	neg.
140	F	60	10.8	29.2	10.9	30.3	pos.

* pregnant

b. Folic acid and vitamin B 12.

In 17 females (3 pregnant) and 1 male, determination of folic acid and vitamin B 12 was made. 9 of them were anemic and 9 non-anemic. In all cases the results were within normal limits (in this laboratory the normal limits for vitamin B 12 are 151—900 pg/ml and for folic acid 3.1—15 ng/ml).

c. Protein-calorie malnutrition.

In the assessment of the nutritional status in community surveys of this type, the weight/age ratio has been recommended (65) as the most simple method, giving a satisfactory indication of the prevalence of protein-calorie malnutrition.

Although it is important to keep in mind that there are multiple factors involved in producing a poor weight development, an attempt was made to correlate the weight/age ratio to hemoglobin values in children aged 0—14 years. An indication of the general weight development is given in Fig. 20. As there is yet no local reference standard, the Harvard standard was used for the ages 0—59 months and thereafter the Iowa standard (see Jelliffe (65) for references). For each child the weight/age was expressed as per cent of this standard, as suggested by Jelliffe (65), and the means calculated. The standards differ slightly causing a jump after 5 years.

During the first half year the weight/age ratio was close to the American standard (97 %). Although the number of children in this group was small, 11, the finding was consistent with the widely recognized fact that weight development is satisfactory during the first 4—6 months in developing countries, the time when breast milk alone is adequate food and the rate of infection still fairly low. After 6 months, due to inadequate food and a stream of infections, the physical development starts to lag behind. The mean weight/age during the second half year in the Ijaji children was down from 97 % to 80 % and remained at about this level throughout childhood.

In Fig. 25 the mean Hb value for each weight/age group has been calculated for the different age groups and also the percentage of anemia in each of these groups.

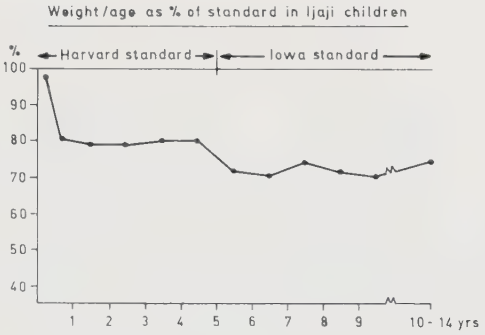

Fig. 20. Mean weight/age ratio, expressed as per cent of Harvard and Iowa standards in Ijaji children. See text.

Fig. 21. "Less than 70 % weight/age".

Fig. 23. Moderate protein malnutrition. Note muscular hypotonia.

Fig. 22. Moderate protein-calorie malnutrition. Note swollen lymph glands in the groins caused by "chiggers".

Fig. 24. "More than 90 % weight/age".

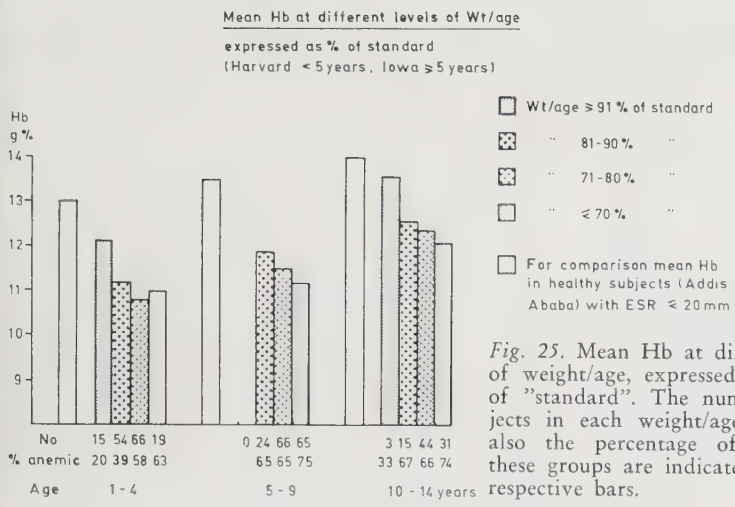
Mean Hb at different levels of Wt/age
expressed as % of standard
(Harvard < 5 years, Iowa ≥ 5 years)
Hb
g %
14
13
12
11
10
9
Wt/age ≥ 91 % of standard
" 81-90 % "
" 71-80 % "
" ≤ 70 % "
For comparison mean Hb in healthy subjects (Addis Ababa) with ESR ≤ 20 mm
No 15 54 66 19
% anemic 20 39 58 63
0 24 66 65
65 65 75
3 15 44 31
33 67 66 74
Age 1-4 5-9 10-14 years

Fig. 25. Mean Hb at different levels of weight/age, expressed as per cent of "standard". The number of subjects in each weight/age group and also the percentage of anemia in these groups are indicated below the respective bars.

In the group less than 1 year the number of children in each weight/age group was small. Most children aged less than 6 months were found in the weight/age group ≧91 %, and as has been shown earlier a higher mean Hb value was noted at this age as compared to the second half year of life. Infants are therefore excluded from this table.

In the age group 1—4 years the differences in mean Hb between the highest and lowest weight/age group was highly significant. In the group 5—9 years there was no child at all in the weight/age group ≧ 91 %. The difference in mean Hb between the next best weight/age group and that ≦70 % was not significant. In the 10—14 year age group, finally, the difference was almost significant. There were, however, only 3 children in the best group.

It should also be noted that with decreasing mean Hb the percentage of anemic subjects in the lower weight/age groups consequently increased.

The gap in mean Hb from the healthy children in Addis Ababa should be noted, although the 3 children of 10—14 years in the best weight/age group were equal to their Addis Ababa mates when the difference in altitude was taken into consideration.

"Non-infected children".

As was discussed previously a great number of the children presented symptoms and signs of infections of various kinds and the ESR was found to be increased in the majority of cases. It is assumed that many of these infections, especially when combined, or when severe or chronic, cause a decrease in hemoglobin concentration or a slight to moderate anemia (17). In the following an attempt is made to study separately those children who on clinical examination showed no symptoms or signs of infection.

As is indicated in Table 20 this was a comparatively small percentage. It should, however, be pointed out that children with conjunctivitis (acute or chronic as well as trachoma cases), with nasal discharge, and with gum affections have not been excluded but are to be found among the "non-infected children". Children with slight fungal affection of the scalp are also included. Bilateral lymph gland enlargement on the neck and in the axillae and groins were classified in the clinical examinations as + (slight) for pea-bean size, ++ (moderate) for bean-plum size, and +++ (severe) for more than plum size. Any palpable enlargement of more than pea size at least in the axillae and groins

should be considered pathological and, at least under the prevailing conditions, should indicate the presence of or recently passed infection in the respective drainage area. Children with a total lymph gland score of ++ or more were arbitrarily included in the "infected" group.

In the vast majority of cases the children had combinations of various infectious diseases such as a history of diarrhoea, and "chiggers" with lymph gland enlargement.

Not a single child would have been classified as "non-infected" if the signs mentioned above such as conjunctivitis and running nose had also been included.

As the adults underwent a different, abbreviated form of examination, not fully comparable with that used for children and as it was not possible to examine them undressed, only children will be discussed here.

Children 0—11 months. 14 of 33 children were classified as "non-infected" (42.4 %). The ESR was, however, above 20 mm in 8 of these cases (range 22—38 mm).

7 children were below 6 months of age. At least 1 was anemic (Hb 9.5 g %). This child was 2 months old and had a weight/age ratio of 102 % but an ESR raised to 35 mm, possibly indicating a passed or undiagnosed present infection.

7 children were 6—11 months old. If the WHO anemia limit, adjusted for altitude, was applied (Hb less than 11.3 g %), 6 of them would be anemic with Hb ranging between 6.3 and 11.1 g %. The child with Hb 6.3 g % was 11 months old and had a weight/age ratio of 80 % and signs of wasted muscles. He was getting only breast milk and cow's milk (like most of the others in this group) and had a clear iron deficiency anemia (MCHC 21.0 %). All the "non-infected" children in this age group had an MCHC below the mean for healthy children in Addis Ababa, although only 2 had below 27 %. 3 had rickets.

Children 1—4 years. 20 of 161 children were classified as "non-infected" (12.4 %). The ESR was above 20 mm in 13 cases (21—45 mm). 7 were classified as anemic and 10 non-anemic. The Hb value ranged from 8.4—15.3 g %. In 3 cases Hb was not recorded. The mean weight/age was about 80 % with a range of 67—101 %. In no fewer than 8 cases, clinical signs of malnutrition (thin or wasted muscles, dyscolored hair) were recorded and one child was in a state of pre-kwashiorkor — an 18-months old boy with Hb 10.8 g % and ESR 4 mm. The other 6 cases of anemia could be attributed to hookworm (1 case), dietary iron deficiency (milk only, 2—3 cases) and subclinical infections as indicated by raised ESR (32—42 mm).

4 of the 5 children for whom Hb was recorded and with ESR $\leq$20 mm were not anemic. The 5th was the child referred to above with pre-kwashiorkor.

Children 5—9 years. 7 of 163 children were classified as "non-infected" (4.3 %). The ESR was above 20 mm in 6 cases (range 25—39 mm). 4 children were anemic and 3 non-anemic (Hb range 8.8—14.3 g %).

The weight/age ratio was lower than in the younger children, probably due to the use of a different standard, and ranged from 61—79 %. However, none showed any clinical hair or muscle signs of malnutrition and none showed a positive stool test for hookworm (5 tested), although one anemic child with Hb 8.8 g % and MCHC 24.1 % was positive in 2 subsequent stool tests. With the exception of this child none was severely anemic.

Children 10—14 years. 16 of 97 children were classified as "non-infected" (16.5 %). 7 of 15 had an ESR above 20 mm, with a range of 22—50 mm (*sic!*). In one case ESR was not recorded. 9 children were anemic and 6 non-anemic (no Hb record in one case). The Hb ranged between 10.8 and 14.8 g %.

The weight/age ratio varied considerably (48—97 %), probably due in part to unprecise age information. One child had dyscolored hair but no other signs of protein malnutrition and 2 had hookworm, one of whom showed signs of iron deficiency anemia (Hb 10.8 g %, MCHC 24.5 %).

Of the 7 children with ESR ≤20 mm, 3 were anemic — one was the child referred to above with hookworm, the other 2 were marginally low.

Children with weight/age ratio above 90 %. In the age group 1—4 years the mean weight/age ratio was stable at about 80 % of the Harvard standard (see Fig. 20). The age determination can be considered satisfactory as the vast majority were examined several times after the base line examination and each time an attempt was made to arrive at an even more exact age, utilizing a local calendar. Before 1 year nearly all children dropped successively in weight/age ratio and after 5 years the age determination started to be more difficult. For these reasons only the children of ages 1—4 years with a weight/age ratio of more than 90 % of the standard were selected for consideration. Only 15 out of 154 (9.7 %) blood-tested children then remained (see also Fig. 25). Their weight/age ratio ranged between 91 and 116 %.

12 were non-anemic (Hb 11.3—15.3 g %) and 3 anemic (Hb 8.7—11.1 g %). Even if these absolute figures are small, the rate is considerably lower than in the total group (20 and 48 % respectively). The anemia could be ascribed to hookworm in 2 cases while in the third child there was no stool record. All 3, however, also had signs of infections according to previously discussed criteria; wasted muscles and moderate liver enlargement were noted in 2 of the anemic children.

Of the 12 non-anemic children only 4 had "no infection", although in 3 of these 4 the ESR was above 20 mm (21—28 mm). The "infected" non-anemic cases had ESR values between 15 and 45 mm.

Serum iron and transferrin. The result of SI and TIBC determinations in children 5—14 years (55 boys and 21 girls) and in adults are given in Table 23.

Table 23. *Results of determinations of Hb, ESR, SI, TIBC and per cent transferrin saturation in children and adults in Ijaji.*

Age group		Hb*	ESR*	SI	TIBC	% satur.
5—14 years	Mean	12.6	25.4	65	314	20.8
	S.D.	± 1.02	± 9.95	±28	± 46	± 8.5
	No.	76	75	76	76	76
Adult males	Mean	13.6	17.2	78	282	29.0
	S.D.	± 1.66	±10.7	±33	± 51	±12.1
	No.	38	38	53	53	53
Adult females	Mean	12.1	25.8	68	288	24.0
	S.D.	± 1.54	±11.9	±32	± 43	±11.1
	No.	33	33	53	53	53

* refers to subjects where SI and TIBC were determined.

The mean value for SI in children was markedly below that of healthy children in the same age group in Addis Ababa (65 and 93 µg %, respectively, a highly significant difference) while the mean TIBC values agreed well.

Adult males showed an even greater and highly significant difference in mean SI from healthy and apparently healthy subjects in Addis Ababa, (78 and 125 µg %, respectively) with nearly the same mean TIBC.

The difference in SI for adult females was smaller (68 and 94 µg %, respectively), although also highly significant.

52.7 % of the boys and 52.4 % of the girls, or a total of 40, were anemic. The mean value for SI in the anemic children was 53 µg % and that in the non-anemic group 78 µg %. The difference is highly significant. The mean value for TIBC was similar in both groups — 313 µg % in the anemic group and 316 µg % in the non-anemic group.

None had severe anemia and none displayed the typical picture of severe iron deficiency anemia with marked hypochromia (MCHC below 27 %) low SI and high TIBC.

Of the adults, there was no significant difference in mean SI in males between anemic (n = 15) and non-anemic (n = 23) subjects (77 and 79 µg %), while in the females there was a significant difference (57 and 83 µg %, n = 24 and 9, respectively). The mean values for TIBC were very similar for all the four groups; in anemic and non-anemic males these values were 279 and 280 µg % and in females 283 and 295 µg %.

The high mean ESR should be noted. ESR above 20 mm was recorded in 72.0 % in the children, in 28.9 % in the adult males and in 69.7 % in adult females. In children as well as adults, the mean SI was higher in the groups with ESR ≤20 mm, although the differences were not significant (in children 74 and 62 µg %, in adult males 82 and 68 µg % and in adult females 77 and 59 µg %).

There was no significant difference between SI in subjects with and without hookworm. In children with hookworm (n = 15) the mean SI was 64 µg % and in those without (n = 31) it was 61 µg %. In adults (males and females) with hookworm this value was 61 µg % (n = 9) and in those with a negative stool result 81 µg % (n = 43).

Pregnant females.

At the clinical examination all females were asked whether they were pregnant. Some females in the early stages of pregnancy may have concealed their condition or not been aware of it, and may thus have been included in the non-pregnant group.

It was not possible to obtain any exact gestational age in the 14 pregnant females but all were most certainly in the second and third trimester. In 10 of them Hb and PCV determinations were made. Only 1 of these was anemic (Hb 6.8 g %, MCHC 22.7 %). She responded by a moderate improvement after iron injections (see above) but probably did not get adequate amounts of iron for a sufficiently long period. Her serum folic acid and vitamin B 12 values were within the normal range. No stool was given for hookworm examination.

The others ranged between 11.6 and 13.7 g % with a mean value of 12.6 ± 0.87 g % which in fact is the same as for the non-pregnant females in Ijaji. The mean value, including the anemic woman, was 12.0 g %.

The mean SI was 98 ± 33 µg % (n = 11) — the anemic woman had no SI or TIBC determinations. Although the material is small it should be noted that the mean SI was well above that of both males and non-pregnant females in Ijaji and was at the same level as in non-pregnant apparently healthy females in Addis Ababa (94 µg %).

Comments

It was considered useful and necessary to present this material in some detail to give an indication of the complexity of the situation. Compared with "Western standards" very few of the subjects in such a community are "healthy" in the strict sense. The causative factors are multiple and not easy to differentiate when evaluating the role of each factor in, for instance, the etiology of anemia.

In the following, however, an attempt will be made to discuss briefly some of the more important factors contributing to the high degree of the usually mild to moderate anemia found in these investigations.

It was not possible in the field to make red blood cell counts and the only index available was MCHC. Garby *et al.* (32) have stressed that this index is an insensitive method for diagnosing mild form of iron deficiency anemia. The reported "normal value" for MCHC varies a great deal, probably depending on the techniques used (32, 79, 109, 123) but it has the advantage of not being influenced by altitude (57). It may seem inappropriate to select only those anemic cases who had an MCHC below the mean minus 2 SD in the "normal material" in Addis Ababa, for the diagnosis of hypochromic iron deficiency anemia. However, in view of the satisfactory iron depots found in the bone marrow hemosiderin study it must be reasonable to assume that this criteria would give a rough idea about how much iron deficiency contributes to the overall percentage of anemia. It has been pointed out that this type of anemia is the predominant one in the tropics (62, 88), forming a formidable public health problem. The index MCHC has been used in this study, utilizing the "normal material" in Addis Ababa as a reference standard and with a liberal range (mean — 2 SD), thereby possibly underestimating the frequency of iron deficiency anemias.

The anemia of infection is usually normochromic but may be hypochromic, although the hypochromia is not as marked as in iron deficiency anemia (17).

If the criteria for iron deficiency anemia mentioned are used it would be resonable to assume that a conservative estimation of this type of anemia could be made. The overall rate was 16.9 % — or roughly one sixth of all anemias would be of the iron deficiency type.

There was, however, a general tendency to hypochromia in Ijaji compared to the healthy material in Addis Ababa (Table 18), most marked in the small children. The overall rate of marked hypochromia (MCHC <27 %) in subjects more than 1 year of age was 9.1 % compared to 3.3 % in Addis Ababa. This should be matched by the rate of MCHC >35.0 % or mean + 2 SD for Addis Ababa. This figure was 2.6 % in Ijaji and 2.3 % in Addis Ababa (cf. Figs. 13 and 19). In other words, marked hypochromia was about 3 times more common in Ijaji.

The fact that the highest rate was found among small children (Fig. 19) agrees with the food pattern, providing a low dietary iron intake during the first 1½—2 years when the diet consists mainly of milk and starchy gruels (101). After this age, the children start to participate in the adult (iron rich) meals and the hypochromic anemia seen in older children and adults is presumably caused to a larger extent by iron drain rather than low intake. It would be reasonable to assume also that the high frequency of diarrhoeal diseases, striking hardest the infant and pre-school groups (Table 20) would also contribute to an impaired iron absorption during this period of limited intake and rapid growth.

No chemical studies have been performed regarding the intake of folic acid (and vitamin B 12), although green leafy vegetables, for instance kale, is consumed and also, though not too frequently, meat (101). The fact that none of the 18 subjects examined had abnormal serum folic acid and B 12 levels does not exclude the existence of megaloblastic anemia. This type of anemia is more common in fe-

males and especially in pregnant females. The fact that 17 of the tested subjects were females, 9 of whom were anemic and 3 pregnant, one with a severe anemia, may indicate that if folic acid or B 12 dependent anemias exist they may not constitute a major problem. Further studies in this field are desirable, however.

The role of protein-calorie malnutrition *per se* in the etiology of anemia is very difficult to evaluate. In Rhesus monkeys fed a low protein diet (105) a normoblastic, normochromic anemia developed, as well as a marked reduction in the serum iron, transferrin, albumin and the total serum proteins. An interesting point was that the gamma globulins showed an increase. All these changes could be reversed by re-feeding, although the gamma globulins remained high. During the time of the low protein diet the animals also had a reduced iron absorption. The anemia was moderate in contrast to the severe reduction in albumin and the marked atrophy of organs with a high protein turnover, indicating that hemoglobin synthesis was maintained at the expense of other body proteins.

A reduced erythroid activity in the bone marrow in animal studies (105) as well as in patients with severe protein-calorie malnutrition (34, 103) has been postulated as the cause, resulting in a decreased cellular proliferation and interpreted (103) as an expression of adaptation to undernutrition. However, the anemia of kwashiorkor may have a multiple etiology or the etiology may be dependent on a locally prevailing deficiency pattern (28, 34, 90). With protein feeding, associated deficiencies may become apparent or more marked (34).

In the present study, the children did show a low weight/age ratio and also a low weight/height ratio and in about 1/3 of them there were clinical signs of protein malnutrition, mainly thin or wasted muscles and thin or dyscolored hair but only occasionally was frank kwashiorkor or marasmus seen (56).

The weight/age ratio in the blood-tested "privileged" children in Addis Ababa was in the pre-school group slightly above 90 % and after that about 85 %, thus about 10 % higher than in Ijaji but still not "optimal", although none showed any clinical signs of protein malnutrition.

The significant differences in mean Hb between the highest and the lowest weight/age groups (1—4 and 10—14 years, Fig. 25) do not necessarily indicate a relationship between protein malnutrition *per se* and low hemoglobin concentration but may merely indicate that a number of factors which cause a poor weight development, such as chronic or frequently recurring infections, at the same time cause a decreased Hb or frank anemia. The fact that the children 1—4 years with a weight/age ratio of >90 % had less anemia but still had infections in a high percentage (although less than the whole group) does not necessarily speak against this.

It must be admitted that although there may be some indications that protein deficiency *per se* may play a part in contributing to the generally low hemoglobin levels in Ijaji, its exact role cannot be established.

A total of 17.9 % of the blood-tested subjects ≧1 year for whom stool samples were examined were positive for hookworm (Fig. 18). In all ages above 1 year the overall rate of anemia in subjects with hookworm was 73 % and in those with no hookworm 55 %. The difference would indicate the percentage of anemia due to hookworm infestation (68). This would be 18 %, which is about 1/4 of all anemias seen in the hookworm positive group. Apparently there remains 55 % anemia to be accounted for.

There were significant differences between mean Hb values in hookworm positive and negative children 1—14 years old but not in adults. The MCHC value was below 27 % in a high percentage (37.5 %) in hookworm positive anemic cases of ages 1—4 years. In older children this percentage was lower and in adults with hookworm none had a value

below 27 %. This indicates that the small children seem to be more vulnerable to the blood loss caused by the worm, than older children and adults.

Some caution may be necessary in interpreting the figures given. Generally, hookworm infestation was more common in the pure farming areas in the outskirts of the village than in the center, although positive cases were seen from all areas. The health condition was less good among the farmers and in particular the rate of infections among their children was considerably higher. It cannot be excluded, therefore, that factors other than hookworm contribute substantially to the higher rate of anemia found among the hookworm positive subjects and that the statement that 1/4 of all anemias found in this group are due to hookworm is an overestimation. However, hookworm seemed to be responsible to a larger extent for the severe anemias. A total of 26 subjects in all age groups had Hb values below 9 g %. In 15 of these, stool samples were examined and 9, or 60 %, were positive for hookworm, which is about 3 times the overall rate.

In comparison with more heavily infested areas in the world (29, 31, 68, 107), the prevalence of hookworm in the Ijaji area is moderate and the degree of anemia encountered must be considered modest. Therapy trials with small amounts of iron in areas heavily infested with hookworm without dewormning (29, 31), have resulted in improved hemoglobin values although the response was even better when antihelminthics were given simultaneoulsy (31). It would therefore seem reasonable to conclude that the high dietary iron intake in this community protects from the development of the more serious and widespread iron deficiency anemias.

There are reasons to believe that chronic or recurring acute infections is the major cause of the anemia diagnosed in this study. It is well known that a mild non-progressive anemia, usually normochromic, not seldom hypochromic, develops as a result of chronic infections and a variety of other chronic disorders (17, 123). Tables 20 and 21 illustrate well the infection situation, and it is probable that the situation is even more serious than what can be revealed in a survey examination, which by necessity must be rather superficial. For instance, cases of primary tuberculosis and a variety of other disorders requiring a more refined set up of diagnostic aids may have been missed. This is also supported by the high number of raised ESR in "non-infected" children. Although the level of ESR does not necessarily completely reflect the state and severity of infection it is widely used and accepted as such an indicator. The relationship between a high ESR and low hemoglobin concentration in this study seems clear (Fig. 17) and cannot possibly be explained by the slightly raised ESR which can be caused by anemia *per se* (123).

The generally low levels of serum iron in Ijaji compared to the healthy subjects in Addis Ababa should also be regarded in relation to the high frequency of infections and high levels of ESR. The mean SI was lower in children and adult males and females with ESR above 20 mm although the difference was not significant. An interesting point is that the mean TIBC values were very similar to those in Addis Ababa. TIBC falls during infections although the reduction is proportionally smaller than that of serum iron (40), and also in states of plasma protein depletion such as in kwashiorkor and nephrosis (8). There was, however, no clinical evidence of marked protein malnutrition in these subjects.

All blood-tested subjects were negative for malaria. Very few cases were diagnosed in the village in 1965—1967, and here the disease was probably brought in from the lowlands; it is possible that the disease will be more prevalent over the next few years with an increasing influx of people from malarial regions.

As far as is known sickle cell anemia has not been diagnosed in central Ethiopia. No investigations have yet been performed in the Ijaji area but this field of hematology deserves penetration.

Summary

A comprehensive investigation of children was made in the village of Ijaji in the fall 1965, later supplemented with children from a nearby village and with adults from Ijaji, all examined during the same season. A separate study on SI and TIBC was, however, made in May (1967).

A total of 726 subjects in ages ranging from 1 month to more than 70 years underwent clinical and hematological examination.

In general, the Hb and PCV values were markedly below those of the healthy subjects in corresponding ages in Addis Ababa and overall the rate of anemia according to current WHO criteria was approximately 60 %.

ESR was markedly increased in the large majority of cases and the mean values in different ages were well above those in the healthy subjects in Addis Ababa. This was found to correspond to a high rate of infection, especially in children, and in a strict sense no child was "uninfected".

SI was significantly lower than in Addis Ababa while TIBC was found to be at the same level.

Serum folic acid and vitamin B 12, determined in a limited number of cases, were within normal limits.

About 18 % of the blood-tested subjects above 1 year of age for whom stool samples were examined had hookworm. Other helminths, although not reported here, were very frequently diagnosed, mainly ascaris.

There was a generally low weight for age ratio in children after 6 months of age. In the age group 1—14 years nearly 30 % were 70 % or less of the "standard".

The anemia was clearly hypochromic (MCHC below 27 %) in a much higher percentage between 6 months and 3 years than after 3 years — 36 and 12 %, respectively, — supporting dietary data, indicating a low iron intake until the children start to participate in the adult, iron rich food. The hypochromic anemia diagnosed in older children and adults was probably due to iron drain rather than a low dietary iron intake.

Reported evidence indicates that hookworm infestation struck hardest in the preschool age group in which the rate of hypochromic anemia in hookworm positive children was highest. Overall about or less than 1/4 of all anemias in hookworm positive subjects seemed to be due to hookworm.

The role of protein-calorie malnutrition in the etiology of the anemia diagnosed is very difficult to evaluate, for reasons discussed. Although the mean Hb decreased with decreasing weight for age ratio, other factors may have been the primary cause of this.

In view of the evidence presented it is concluded that the main causative factor for the generally low hemoglobin values or mild to moderate anemia and the low serum iron values is infections of various kinds. Combinations of different etiological factors are, however, most probably in operation in a major proportion of the subjects studied.

General Summary

Ethiopia may represent the country with the highest daily intake of dietary iron in the world. This is true at least in the highlands, where the grain tef is the staple food. It was therefore considered of interest 1. to study the sources of this iron, 2. to investigate certain hematological values in healthy and apparently healthy subjects of different ages consuming a diet with this high iron content (for adults in the order of 300—500 mg per day), 3. to study the iron stores of different age groups and — for comparison — 4. to investigate certain hematological parameters and the frequency and type of anemia in a representative Ethiopian highland village, in which the iron intake is equally high.

As it was suspected that a substantial part of the iron consumed with the tef was derived from the iron rich soil, 8 tef samples, which had first been cleaned in the traditional way, were further cleaned by washing 20 times in diluted hydrochloric acid. Generally the iron content was thus reduced to between $^1/_2$ and $^1/_4$ of the values obtained after the traditional cleaning, indicating that most of the iron is to be found *on* the tef as contamination but that the iron content *in* the tef would still be 3—4 times that in wheat. This would mean a daily iron intake from the tef grain itself for an adult male of approximately 75—100 mg. If contributions from other food items are included (in the order of 25 mg) the total daily iron intake from the food itself will far exceed recommended allowances and the actual intake in most other countries.

In Chapter III the results of hematological examinations of 615 healthy and apparently healthy subjects of ages 1—65 years are presented. 78 of these were pregnant females. In addition 30 newborn children had capillary blood tests and another 47 had cord blood examined.

In general the mean hemoglobin values in the different age groups agreed well with accepted "standard values" after an altitude adjustment of the latter by +7 %. Also the mean values for SI and TIBC agreed satisfactorily with those of other workers. In pregnant women and delivering mothers there was only a comparatively small decrease in mean Hb values during the last trimester compared to non-pregnant females and no decrease at all in SI while TIBC was raised as is usual in pregnancy. The anemia rate in subjects 1—65 years of age was low, approximately 5—7 %. In adults no certain cases of iron deficiency anemia were diagnosed with the criteria used, while the figure in children was about 3 %.

In Chapter IV the result of an investigation of bone marrow hemosiderin in 136 healthy and apparently healthy subjects of ages 3—65 years indicated an increase of bone marrow iron with increasing age. In subjects 30 years or more, +++ — ++++ hemosiderin (scale 0 — ++++), was diagnosed in about 80 %. All except 3 young children had stainable hemosiderin. In 19 cases with bone marrow hemosiderin of trace — ++++, a desferrioxamine test (500 mg given intramuscularly) was made and 6 hours' iron excretion in the urine determined. Although the iron excretion was higher in the group with hemosiderin +++ — ++++ compared to the group trace — ++, the difference was not significant and there was a considerable scattering of individual values. The highest excretion value was 1.43 mg. For reasons discussed, the findings are interpreted as indicating that in the subjects who underwent bone marrow studies the increased iron was mainly localized reticuloendothelially and that this would represent an early and comparatively mild iron overload state. This is supported by chemical iron analysis of 98 autopsy liver specimens, 76 of which were from subjects who died of trauma (6 females), in which the mean non-hemin iron

values were not significantly raised above those of hematologically normal Swedish males. Although in this material there was a high incidence of suicide and homicide, subjects probably in a less satisfactory nutritional condition, the results indicate nevertheless an adequate or more than adequate iron nutrition.

In Chapter V the results of hematological investigations of 726 subjects, of ages 1 month to more than 70 years, in a representative Ethiopian highland village are presented. No less than 60 % were diagnosed as having anemia, 17 % of which was of the iron deficiency type. The prevalence of iron deficiency anemia was much higher in the ages 6 months to 3 years than after 3 years, when the dietary iron intake is high. About 18 % of the blood-tested subjects above 1 year of age for whom a stool sample was examined had hookworm, but probably less than 1/4 of the anemia cases diagnosed in this group were due to hookworm *per se*. In 18 cases the serum folic acid and vitamin B 12 values were examined and found within normal limits. There was, especially among children, a very high prevalence of infections of various kinds and the majority of the subjects studied had raised ESR. Although protein-calorie malnutrition may have played a part in the etiology of anemia, it is concluded that the main causative factor is repeated acute or chronic infections.

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